INTEGRATION OF A COMBINED UASB-OZONATION TREATMENT SYSTEM FOR CELLAR EFFLUENT DEGRADATION

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

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ABSTRACT

The wine industry significantly contributes to South Africa's water demand and subsequent pollution of the limited resource. Wastewater is produced throughout the year with an increase in volume and organic load during the vintage season. Anaerobic digestion (AD), specifically the upflow anaerobic sludge bed (UASB) technology has been shown to be feasible in the treatment of cellar wastewater. However, the legal standard for chemical oxygen demand (COD) for disposal in a natural water resource (75 $mg.L^{-1}$) is often not met. The aim of the study was to conduct a laboratory-scale investigation into the feasibility of combining pre- and post-ozonation processes with AD in order to achieve a final COD closer to the legal disposal limit.

While acclimatising an UASB bioreactor containing mixed anaerobic granules to a cellar wastewater with a pH set at 8.0, stable-state conditions were not reached. Sucrose additions to the substrate, increased substrate loads, heat-treatment of the substrate and an addition of isolated cellar effluent bacteria to facilitate degradation prior to AD, were all unsuccessful in maintaining stable-state in terms of COD removal efficiency. Once the substrate pH was re-set to 7.5, the reactor stabilised. The lowest efficient operational pH was found to be 5.73 resulting in a COD removal of 88% at a substrate COD < 5 000 $mg.L^{-1}$. At a substrate pH of 6.0, the lowest efficient operational hydraulic retention time (HRT) and corresponding organic loading rate (OLR) were 19.7 h and 9.75 kg $COD.m^{-3}d^{-1}$, respectively, with the COD removal being maintained around 84%. The reactor effluent still had a final COD of 1280 $mg.L^{-1}$, which was well above the legal South African limit.

Dominant bacteria were isolated from raw cellar wastewater and identified as *Acinetobacter haemolyticus*, *Burkholderia cepacia* and *Cryseomonas luteola*. In order to investigate the possibility that ozonation improved biodegradability, the growth of the isolates at 35°C was monitored over 24 h in sterile ozonated and non-ozonated substrates from the vintage and non-vintage seasons. All the isolates increased by at least 1.5 log cycles in the control substrates from both seasons. Ozonation of the wastewater batches for 10 min at a rate of 73 $mg.L^{-1}$ led to slightly increased growth of the inoculants in the substrate batch from the

vintage season. For the substrates from the non-vintage season, ozonation had an inhibitory effect on the bacterial growth.

A 5 min ozonation treatment at a concentration of 73 $mg.L^{-1}$ was found to be optimal for both a pre- and post-treatment to UASB-treatment of cellar wastewater. Both UASB treatment and ozonation were effective in reducing the COD by 85% and 20%, respectively. The COD reduction was improved to 88% when UASB treatment was combined with post-ozonation. The total reduction in total suspended solids (TSS) for the combined process was 97%, compared to 80% for UASB and 73% for an ozone treatment alone. The reduction for volatile suspended solids (VSS) was 98% compared to 81% for UASB and 73% for the ozone treatment alone. The total reduction when using a pre-ozonation UASB treatment combination was an average of 86% for COD. The TSS and VSS were both reduced by 95%. Biogas production increased from 1.4 $L.d^{-1}$ to 3.8 $L.d^{-1}$ when an ozonated wastewater was used as substrate. When the UASB treatment was combined with both a pre- and post-ozonation treatment process, the COD was reduced by 89% while TSS and VSS were both reduced by 99%.

This study showed that pre- and post-ozonation treatment processes could successfully be utilised to improve UASB treatment of cellar wastewater. Although the legal limits for discarding into a natural resource were not met, significant progress was made in reducing COD levels. Cellar wastewaters do however, vary according to season and the wastewater composition could affect the efficiency of a pre-ozonation process.

UITTREKSEL

Die wynindustrie maak 'n beduidende bydrae tot die eise wat aan Suid-Afrika se waterbronne gestel word en gevolglik die besoedeling van die beperkte hulpbron. Afloopwater, wat in volume en organiese lading gedurende die parstyd toeneem, word reg deur die jaar opgelewer. Anaërobiese vertering (AV), spesifiek die "Upflow anaerobic sludge blanket" (UASB) tegnologie, is alreeds suksesvol gebruik om kelderafloop te behandel. Die wetlike vereiste vir chemiese suurstof behoefte (CSB) vir storting in 'n natuurlike hulpbron (75 $mg.L^{-1}$), word egter dikwels nie bereik nie. Die doel van die studie was om in 'n laboratorium-skaal ondersoek AV te kombineer met voor- en na-osoneringsprossesse, om sodoende te poog om 'n CSB nader aan die wetlike standaard te verkry.

Terwyl 'n UASB bioreaktor wat gemengde anaerobiese granules bevat het, geakklimatiseer is tot kelderafloop met 'n pH gestel tot 8.0, kon stabiele toestande nie bereik word nie. Die byvoeging van sukrose tot die substraat, verhoogde substraatladings, hitte-behandeling van die substraat en die byvoeging van geïsoleerde kelderafloop bakterië om substraatafbraak voor AV aan te help, was onsuksesvol om stabiliteit in terme van CSB-verwydering, te handhaaf. 'n Verstelling van die substraat pH na 7.5, het gelei tot reaktorstabiliteit. By die laagste doeltreffende bedryfs-pH van 5.73 en substraat CSB < 5 000 $mg.L^{-1}$, was die CSB-verwydering 88%. By 'n substraat pH van 6.0 was die laagste doeltreffende bedryfs-hidroliese retensie tyd en -organiese ladingstempo 19.7 h en 9.75 kg $CSB.m^{-3}d^{-1}$, onderskeidelik, terwyl die CSB verwydering rondom 84% gehandhaaf is. Die CSB van die reaktoruitvloesel van 1 280 $mg.L^{-1}$, was steeds ver bo die wetlike vereiste.

Dominante bakterië is uit kelderafloop geïsoleer en as *Acinetobacter haemolyticus*, *Burkholderia cepacia* en *Cryseomonas luteola*, geïdentifiseer. Die moontlikheid dat osonering bioafbreekbaarheid bevorder, is ondersoek deur die groei van die isolate by 35°C oor 24 h in steriele geösoneerde en ongeösoneerde substrate te monitor. Die substrate is berei vanaf kelderafloop wat in die parsseisoen sowel as die nie-parsseisoen versamel is. Al die isolate het met ten minste 1.5 log siklusse in die kontrole substrate van beide seisoene, vermeerder.

Vir die kelderafloop wat in die parsseisoen versamel is, het osonering vir 10 min teen 73 $mg.L^{-1}$ gelei tot effens verbeterde groei van die innokulante. Osonering het 'n onderdrukkende effek op die groei van bakterië in die afloopwater versamel in die nie-parsseisoen, gehad.

Osonering vir 5 min teen 'n konsentrasie van 73 $mg.L^{-1}$ is as optimum vir beide voor- en na-osoneringsbehandeling tot UASB-behandeling van die kelderafloop, gevind. UASB-behandeling en osonering het die CSB met 85 en 20% onderskeidelik, verminder. Die vermindering kon tot 88% verhoog word wanneer UASB-behandeling met na-osonering gekombineer is. Die vermindering in totale gesuspendeerde vastestowwe (TGV) vir die gekombineerde proses was 97%, in vergelyking met 80% vir UASB- en 73% vir osoonbehandeling alleen. Die vermindering in vlugtige gesuspendeerde vastestowwe (VGV) was 98% in vergelyking met 81% vir UASB- en 73% vir osoonbehandeling alleen. Die totale CSB verwydering vir 'n voor-osonerings UASB kombinasie was gemiddeld 86%. Die TGV en VGV is beide met 95% verminder. Biogasproduksie het ook vermeerder vanaf 1.4 $L.d^{-1}$ tot 3.8 $L.d^{-1}$ toe geösoneerde afloopwater as substraat gebruik is. Die kombinasie van UASB-behandeling met voor-osonering, sowel as na-osonering het gelei tot 'n CSB-verwydering van 89% terwyl TGV en VGV beide met 99% verminder is.

Hierdie studie het getoon dat voor- en na-osonering suksesvol gebruik kan word om UASB-behandeling van kelderafloop te verbeter. Hoewel wetlike vereistes vir storting in 'n natuurlike hulpbron nie bereik is nie, is beduidende vordering gemaak in die verlaging van CSB-vlakke. Die verskil in die samestelling van kelderafloop gedurende die onderskeie seisoene, kan egter die doeltreffendheid van die voor-osoneringsproses beïnvloed.

dedicated to my parents

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CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

South Africa has a serious water limitation and that which is available must supply the needs of households, the mining, agricultural and industrial sectors (Republic of South Africa Presidents' Council, 1991). Pressure is increasingly being placed on the water resources by the rising living standards of the majority of South Africans and the development of the industrial sector. The average water consumption *per capita* in underdeveloped areas is 20 $L.d^{-1}$, and it is expected that this figure will increase to 300 $L.d^{-1}$. In 1990, industry accounted for 7% of South Africa's total water demand and the predicted percentage for 2010 is 11.4% (Yannakou, 1997). It is estimated that water demand in the Western Cape region could increase by between 0.7 and 2.8% per annum, depending on the management of the resource (New, 2002). Thus, to assure adequate water for the future it is important to restrict pollution to a minimum and reuse effluents to supplement the limited freshwater resources. Waste treatment must thus be a priority (Department of Water Affairs and Forestry, 1991).

The wine industry contributes to "water demand" and the increased wine production over the last decade has led to even more pressure being placed on South Africa's limited water resources (Van Schoor, 2000). Between 700 and 3 800 L of water per ton grapes processed or 0.8 to 4.4 L per litre of wine produced, are generally used (Water Research Commission, 1993). Bottling operations lead to further water requirements and subsequent increased volumes of polluted wastewater.

In the cellars *ca.* 50% of the water is used for cooling of fermentation cellars and/or vessels to around 15°C (Water Research Commission, 1993). The other 50% is used for the washing of vessels, equipment and floors. On average, 70% of the water taken in becomes a source of polluted wastewater. As batch processing is practised and cellar activities differ during the vintage and non-vintage seasons, the wastewater quality shows significant daily and seasonal variation in pollution load. However, due to on-going cellar activities, wastewater

with a chemical oxygen demand (COD) of 800 to 12 800 $mg.L^{-1}$ (Petruccioli *et al.*, 2002), is produced throughout the year (Bezuidenhout *et al.*, 2002).

Irrigation is currently the preferred method for cellar wastewater disposal (Bezuidenhout *et al.*, 2002). To irrigate less than 500 $m^3.d^{-1}$, the wastewater should have a COD of less than 400 $mg.L^{-1}$. To dispose of the wastewater to a natural resource, the COD must be lower than 75 $mg.L^{-1}$ (Anon., 1999). Treatment prior to disposal is thus a necessity. Furthermore, public concern for the environment, which has led to stricter legislation and higher penalties for non-compliance, and the wish to stay competitive in an increasingly globalised market place (Hayward *et al.*, 2000), has forced cellars to consider and implement on-site wastewater treatment options.

It is well known that biological treatment methods are especially feasible in the treatment of wastewaters with high organic contents (Benitez *et al.*, 1999). The aerobic treatment processes, although relatively effective, have the disadvantages of having high operating costs and generating large volumes of biomass (sludge), which must be disposed of in an environmentally friendly manner. In comparison, anaerobic digestion (AD) produces three to twenty times less biomass, and uses readily available CO_2 as electron acceptor (Bitton, 1999), eliminating the high costs of sludge disposal and aeration. Anaerobic digestion also produces methane, which can be used as energy source to produce hot water or steam to maintain the anaerobic bioreactor's temperature. The total energy required for wastewater treatment is thus reduced. Higher influent organic loading is possible (Shieh & Nguyen, 1997), as the process is not limited by the oxygen transfer capability at high-oxygen utilisation rates as found in the aerobic process. While aerobic treatment requires relatively high levels of nutrients, mainly phosphorus and nitrogen, AD can precede at low levels of these nutrients (Ditchfield, 1986). Furthermore, hazardous organic wastes that are resistant to aerobic breakdown can usually be treated anaerobically (Nazaroff & Alvarez-Cohen, 2001).

A specific application of AD, namely the upflow anaerobic sludge blanket (UASB) technology, developed by Lettinga and co-workers in the 1970s (Lettinga & Hulshoff Pol, 1991) offers several advantages over other anaerobic digestion processes. The UASB system does not have the clogging problems of attached growth systems or the high-energy requirements of fluidised bed reactors (Lin &

Yang, 1991). It also has the advantage of biomass retention within the system by granulation of the anaerobic bacteria. This leads to high removal efficiencies at high volumetric COD loading rates and short hydraulic retention times (HRT) (Hickey *et al.*, 1991; Schmidt & Ahring, 1996). Upflow anaerobic sludge blanket systems have successfully been employed for the treatment of various wastewaters from the food industry. These include maize, meat and dairy processing, brewery, fruit cannery and cellar wastes (Ross, 1989; Strydom *et al.*, 1997; Puñal & Lema, 1999; Ronquest & Britz, 1999; Trnovec & Britz, 1999; Sigge *et al.*, 2002). Using UASB technology, Müller (1998) found the removal efficiencies for BOD and COD of a cellar wastewater to be more than 90% at a starting COD of 941 to 13 600 $mg.L^{-1}$. Similarly, Ronquest & Britz (1999) have shown that more than 93% COD reductions could be achieved when treating cellar wastewater at an organic loading rate (OLR) of 10.1 $kgCOD.m^{-3}d^{-1}$, a HRT of 14 h and a reactor influent pH of 5.1.

Often the required goals of wastewater treatment cannot be reached by using only one treatment method. In these cases combined strategies, biological and chemical treatment methods may often be utilised (Gottschalk *et al.*, 2000). These types of combinations use the strength of each process and accordingly could lower the size of the unit and capital costs and could increase efficiency.

Ozonation is an old water treatment technology, already used by some European countries since the beginning of the twentieth century (Yu & Yu, 2001). Since ozone does not leave any residual compounds in the wastewater (Graham, 1997), it may be an excellent option as pre- and post-treatment method to the AD process. As a pre-treatment to AD, ozone makes recalcitrant compounds more biodegradable (Gottschalk *et al.*, 2000). Andreozzi *et al.* (1998) found that phenols and unsaturated lipids, both inhibitory to AD, could be reduced by 50% using an ozone treatment. Ozone has also been shown to increase the methane yield during the AD process (Benitez *et al.*, 1999; Martin *et al.*, 2002). However, oxidation processes such as ozonation could also lead to the formation of reaction intermediates of greater toxicity than the starting substances (Andreozzi *et al.*, 1997; Martin *et al.*, 2002). Ozonation only improves biodegradability until a specific maximum is reached. Upon further ozonation, biodegradability decreases (Gottschalk *et al.*, 2000).

As a post-treatment, ozone has been shown to reduce colour and COD levels (Athanasopoulos & Athanasopoulos, 1998; Gottschalk *et al.*, 2000; Sigge *et al.*, 2001; Sigge *et al.*, 2002). Athanasopoulos & Athanasopoulos (1998) used a post-ozonation treatment to reduce the COD and colour of the effluent from an UASB reactor treating "currant" wastewater by 73 and 74%, respectively. Sigge *et al.* (2002) found that UASB treatment of cannery wastewater (HRT = 24 h, OLR = 7.5 kg $COD.m^{-3}d^{-1}$ and substrate pH = 7.5) could lower the COD by 90 to 93%. A combination with ozonation was found to reduce the COD and colour of the UASB effluent by 27 to 53% and 66 to 90%, respectively. In the same study they reported that an UASB (HRT = 24 h, OLR = 3.7 kg $COD.m^{-3}d^{-1}$ and substrate pH = 7.5) was also able to lower the COD of a cellar wastewater by 90 to 96%. With the implementation of a post-ozonation step, the COD and colour levels were further reduced by between 30 and 55%, and 68 and 87%, respectively. These combination treatments led to final COD values as low as 247 and 67 $mg.L^{-1}$ for the cannery and cellar wastewaters, respectively.

The objective of this study was to investigate the feasibility of combining ozonation as a pre- and as a post-treatment with the anaerobic digestion of cellar wastewaters. The will be done by firstly stabilising and then optimising a laboratory-scale UASB bioreactor treating cellar wastewater in terms of lowest pH and highest OLR under which efficient digestion, specifically in terms of COD removal efficiency, could be achieved. Bacterial growth in ozonated cellar substrates will also be investigated to determine if ozonated cellar wastewaters could be inhibitory to bacterial growth and thus also the AD process.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

Water is the most limiting resource in a semi-arid South Africa, with an average rainfall of less than 500 mm, which is well below the world average of 860 mm (Holtzhausen, 2002). The rainfall is not only limited but also variable and unevenly distributed in a country with few permanent streams and unpredictable extremes in the form of droughts and floods. As rainfall and the relatively small run-off of 32×10^6 m^3 per year, is poorly distributed in relation to the areas of greatest economic activity, water needs to be transported over great distances. The standard of living of the majority of South Africans is currently rising and with this also an increased demand for water (Republic of South Africa President's Council, 1991). It is thus important to restrict pollution to a minimum. The reuse of wastewater is vitally important to supplement scarce freshwater resources and thus waste treatment must be a priority (Department of Water Affairs and Forestry, 1991).

B. POLLUTION

Industries worldwide use about twice the amount of water that households use (Yannakou, 1997). In 1980, South Africa's industrial sector already accounted for 6.3% of the total water demand. In 1990, this rose to 7% and it is expected that this figure will increase to 11.4 % in 2010. Although industry only consumes little more than 10% of the water it withdraws, the fraction that is returned is heavily polluted (Cosgrove, 2002).

The food and beverage industry makes a significant contribution to current pollution. One cannot expect that all individual industries will produce the same quality or quantity of wastewater. However, most food processing wastes do have the following similar characteristics: high concentrations of organic material such as carbohydrates, proteins and lipids; high concentrations of suspended solids;

high biological oxygen demand (BOD) and chemical oxygen demand (COD); high nitrogen concentrations; high suspended oil and/or grease; and large variations in pH (Yannakou, 1997; Sigge, 2000).

Even when just looking at the dairy industry, there is considerable variation in wastewater characteristics. Strydom *et al.* (1997) found the average COD concentration of wastewater for a cheese factory to be 5 340 $mg.L^{-1}$, for a fresh milk factory it was 4 656 $mg.L^{-1}$ and for a milk powder/butter factory it was only 1 908 $mg.L^{-1}$. It was also found that flow-rate, pH and chemical composition of wastewater were highly variable over a time period of 13 h when samples were taken hourly.

A study conducted in 1991 showed that the South African poultry industry consumes approximately 6 million m^3 of water yearly of which about 90% is discharged as wastewater (Skivington, 1991). In large-scale chicken abattoirs (more than 10 000 slaughtered daily) the average water intake per bird is 17 L with 29 g COD being produced per bird. The high COD is attributed to the presence of blood, skin, fat, feathers, viscera and faeces in the wastewater.

The soft drink industry that includes carbonated soft drinks, dairies and fruit juice packaging plants, consumes an average of 4×10^6 L of water yearly (Skivington, 1991). Between 50 and 80% of this water is discharged as wastewater with a "Specific Pollution Load" of 4 000 mg $COD.L^{-1}$.

Another example is the gelatin industry where the production of 1 ton of product requires about 300 tons of water (Hunt, 2000). Again a large portion, approximately 80%, is discharged as wastewater.

The wine industry also contributes to pollution (Van Schoor, 2000). As wine production has increased during the last decade, the pressure on natural resources, specifically water, soil and vegetation, has also increased. This wastewater has the potential to cause salination and eutrofication of water resources. The pollution can even be extended to soil degradation, soil water logging, chemical contamination, erosion and destruction of soil structure.

C. SOUTH AFRICAN WINE INDUSTRY

It has been estimated that 10% of the grape crop is used in the manufacturing of grape juice and concentrates while the rest is fermented to wine (Water Research Commission, 1993). The water usage in winemaking ranges from 700 to 3 800 L per ton of grapes processed, with the most water used for cellar cooling and the washing of floors, vessels and equipment (Water Research Commission, 1993; Bezuidenhout *et al.*, 2002). The bottling process also consumes water with the specific amount being dependent on the type of product produced. The average is 1.5 L per bottle. It has been shown that approximately 70% of the water taken in by a cellar leaves as wastewater (Water Research Commission, 1993). The water needed to produce one bottle of wine varies between 2.5 and 10 L (Bezuidenhout *et al.*, 2002).

Cellar wastewater, a nutrient deficient substrate, is difficult to treat, even more so than sewage (Toffelmire, 1972). Cellar wastes include pomace (dewatered grape skins, seeds and pulp), lees, stillage, bottle washings, spillage waters, cooling waters and salt water from ion-exchange processes. The final wastewater has a fairly high COD varying between 800 to 12 800 $mg.L^{-1}$ (Petruccioli *et al.*, 2002). It also has the tendency to become acid and odorous when left standing (Toffelmire, 1972).

During the vintage period, which lasts between 6 and 20 weeks, grapes are harvested and pressed while the juice is fermented to wine (Van Schoor, 2000). The bulk, generally more than 50% of the wastewater, is produced in the vintage period (Bezuidenhout *et al.*, 2002). Due to the nature of vinification, the wastewater frequently exhibits large daily and seasonal variations. Stabilisation, filtration, maturation, blending and the bottling of wine are the major activities during the non-vintage period (Van Schoor, 2000).

Bezuidenhout *et al.* (2002) audited 14 South African wineries during the 2000 and 2001 vintage seasons. An additional 9 cellars were included in the 2001 audit. A summary of the results is given in Table 1 and an average typical composition of wastewater produced during bottling processes is shown in Table 2 (Storm, 1997).

Table 1. Summary of data obtained in an audit of South African cellar wastewater during the vintage periods 2000 and 2001 (Bezuidenhout *et al.*, 2002)

	2000			2001		
Constituent	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**
Chemical oxygen demand ($mg.L^{-1}$)	2 796	33 180	12 972	1 401	19 754	8 664
Total dissolved solids ($mg.L^{-1}$)	1 186	11 424	3 381	618	4 275	2 537
Suspended solids ($mg.L^{-1}$)	223	18 806	2 406	232	7 478	1 511
pH	305	5.6	4.2	3.5	8.8	4.6
Conductivity ($mS.m^{-1}$)	4	230	93	19	253	105

Table 2. Composition of the waste stream generated by a large operating winery during bottling operations (Storm, 1997)

Constituent	Concentration ($mg.L^{-1}$)
Chemical oxygen demand (COD)	9 330
Biochemical oxygen demand (BOD)	5 845
Total suspended solids (TSS)	1 251
Ammonia nitrogen (NH_3)	14.6
Volatile suspended solids (VSS)	722
Total nitrogen (N)	81
Total dissolved solids (TDS)	178
Total phosphorous (P)	8.8
Settleable solids	2.1
pH	5.1

Monitoring, inspection, auditing and policing structures, including the determination of the amount of water consumed, the amount of wastewater generated and the pollution load should be followed in wine cellars to ensure continuing compliance to legislation (Bezuidenhout, 2002). Legislation also requires the analysis of electrical conductivity, pH, sodium adsorption ratio and COD by an accredited laboratory (Van Schoor, 2001). All these analyses are not only required for environmental purposes but are also necessary to remain competitive in an increasingly globalised market place (Hayward *et al.*, 2000).

D. WINE AND DISTILLERY INDUSTRY TREATMENT OPTIONS

There are a wide variety of wastewater treatment methods available to the wine and distillery industries. As treatment is often expensive, it is important to structure the selected procedures in the most effective and economic manner (Hayward, 2000). Options to consider include physical, physio-chemical, biological and chemical methods.

Physical treatment

Sedimentation – Sedimentation basins, a synonym for settling tanks or clarifiers, use gravity to separate solid particles from wastewater (Nazaroff & Alvarez-Cohen, 2001). The wastewater slowly moves horizontally through the basin and material denser than water settles to the bottom where it can be removed. In a similar manner, material less dense than water, such as oil, rises where it can be skimmed from the water. Sedimentation is more often employed as a pre-treatment (Green & Kramer, 1979).

Filtration through granular media – Filtration is specifically applied to remove suspended solids smaller than 10 µm in diameter (Nazaroff & Alvarez-Cohen, 2001) and precipitated phosphorous (Tchobanoglous & Burton, 1991). Various filters have been developed over the years. They differ in the type of operation, type of filtering medium used, direction of flow during filtration, the backwashing process (required when pores between particles become clogged or break-through

occurs) and the method of flow rate control (Tchobanoglous & Burton, 1991; Nazaroff & Alvarez-Cohen, 2001).

Membrane separation processes – Membrane separation involve the use of membranes which are defined as thin layers of material that permit the transmission of water at different rates relative to certain impurities. The most popular processes are reverse osmosis, electrodialysis and micro, ultra and nanofiltration (Nazaroff & Alvarez-Cohen, 2001).]

Physio-chemical treatment methods

Coagulation and flocculation – Coagulation involves the reversal of electrostatic charges on colloidal particles, which favours aggregation of suspended solids (Green & Kramer, 1979). Usually coagulating agents are added in a rapid mixing system (coagulation), which is followed by slow mixing and eventually, sedimentation (flocculation).

Coma *et al.* (1998) evaluated the use of chitosane as flocculant for the pre-treatment of cellar wastewater. The subsidiary sedimentation did lead to a suspended solid and COD reduction of 98 and 40%, respectively. Optimum pH for the process was between 4 and 5, which had the added advantage that it was also in the pH range of the wastewater.

Sorption of organic molecules – Activated carbon is made from carbonaceous materials such as wood, coal, peat, lignin, sawdust and petroleum residues (Green & Kramer, 1979). The material is usually dried at 170°C and then treated in the absence of air at temperatures as high as 600°C to remove volatile organics and to carbonise the organics. A steam or carbon dioxide (CO_2) treatment at 750° to 950°C then activates the carbonised material to open and enlarge the pores.

Activated carbon can be used in granular (GAC) or powdered form (PAC). In the granular form it is usually used in a fixed-bed as filter medium where wastewater is introduced at the top of the column and withdrawn at the bottom (Tchobanoglous & Burton, 1991; Nazaroff & Alvarez-Cohen, 2001). When PAC is used, it is added to the wastewater and after a specific contact period the carbon

is allowed to settle to the bottom of the tank so that the water can be removed (Tchobanoglous & Burton, 1991).

Ion exchange – The ion-exchange process is specifically used for nitrogen and phosphorous removal and demineralisation (Green & Kramer, 1979). It removes cations or anions by replacing them with other ions in a charged resin.

Biological treatments

Activated sludge systems – The activated sludge system (ASS) had its origin in England at the turn of the twentieth century (Bitton, 1999). Degradation of wastewater relies on contact between the organic matter in the wastewater and high concentrations of microorganisms in the presence of dissolved oxygen (Nazaroff & Alvarez-Cohen, 2001). The microorganisms, mostly aerobic heterotrophic bacteria, are suspended within the water of the reactor. These organisms use the organic material for oxidation, producing CO_2 while generating energy for their own biological systems and the synthesis of new biomass.

A typical activated sludge unit consists of a reactor and cell separator. Oxygen (O_2) is supplied to the reactor and this is also where a reduction in BOD occurs. The supply of O_2 does have a dual function. It is firstly used to maintain the microbial life and, secondly, used to keep the liquor in a mixed regime (Gilde & Aly, 1976; Tchobanoglous & Burton, 1991). The required O_2 can be supplied in the form of relatively pure O_2 or as part of compressed air (Walker, 1976).

The water leaving the reactor basin contains high concentrations of microorganisms from the reactor (Nazaroff & Alvarez-Cohen, 2001). In the cell separator, sedimentation is used to separate the water from the bacterial flocs. The water is then discharged and the microorganisms extracted from the bottom of the cell separator are either returned to the inlet of the reactor or processed as waste sludge.

The efficiency of the process relies on the maintenance of the microbial populations. Bacterial flocs are favoured above individual organisms as they are larger and thus also have better settling abilities (Nazaroff & Alvarez-Cohen, 2001). The flocs usually consist of members of the following genera: *Zooglea*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Bacillus*, *Achromobacter*,

Corynebacterium, *Comomonas*, *Brevibacterium*, *Acinetobacter* and filamentous microorganisms (Bitton, 1999). The excess growth of filamentous organisms and the binding of water can give rise to a bulking sludge with very poor settling abilities (Tchobanoglous & Burton, 1991). The cause of bulking is often related to the physical and chemical characteristics of the wastewater, the treatment plant design and limitations, and plant operation. Bulking sludge is undesirable and must be prevented.

The main disadvantages of activated sludge treatment systems are the large initial capital and operational costs. The operators must also be skilled for optimal operation. A problem with the ASS process, especially for the food industry, is the presence of excessive dissolved solid concentrations in the wastewater, which are often not efficiently removed (Green & Kramer, 1979).

The ASS process has several advantages: it occupies little space while handling high organic loading rates; the operator can exercise control over the process; it can tolerate shock loads; and no odour or fly problems are normally experienced. The activated sludge process is furthermore efficient in BOD, suspended solids (SS) and phosphorous removal and may be very efficient in denitrification. The waste sludge is normally stable and can be put onto drying beds (Green & Kramer, 1979).

The ASS process has been shown to be feasible in the treatment of cellar wastewater. The first system of this type for the treatment of cellar waste was installed at Widmer's Wine Cellars in New York State in 1969 to 1970 (Anon., 1972). The system involved the use of four aeration ponds, which overflowed into two final clarifiers for further solid sedimentation and removal. The effluent that was disposed of into a receiving stream had a final BOD of only 30 $mg.L^{-1}$. This was a 97% BOD reduction of the low strength raw wastewater, which had a 1 000 mg $BOD.L^{-1}$ starting load.

Rice (1978) employed a long-term activated sludge process to treat cellar wastewater that consisted primarily of clean-up water containing grape and wine residues. The complete system included a screen, two activated sludge basins, clarifier, chlorination system, reaeration basin, sludge recycle and sludge digestion basin. The influent had an average COD of 3 529 $mg.L^{-1}$, varying from 1 179 to 8 077 $mg.L^{-1}$ for the non-vintage season and an average COD of 4 054 $mg.L^{-1}$, varying from 654 to 5 304 $mg.L^{-1}$ for the vintage season. With a

retention time ranging from 10 to 20 d in the activated sludge basins, effluents with average CODs of 206 and 92 $mg.L^{-1}$ for the non-vintage and vintage seasons respectively, were generated. The mean COD reduction for the whole season was approximately 95%. The average TSS for the non-vintage season was 364 $mg.L^{-1}$, varying from 40 to 2 252 $mg.L^{-1}$ and for the vintage season an average of 757 $mg.L^{-1}$, varying from 95 to 1 617 $mg.L^{-1}$. The treated wastewaters had an average TSS of 44 and 33 $mg.L^{-1}$ for the non-vintage and vintage seasons, respectively. The average removal of TSS was not efficient enough to meet the permitted discharge levels of 20 $mg.L^{-1}$. The addition of a polymer flocculant at a concentration of 5 to 15 $mg.L^{-1}$ was necessary to reach the set standard.

At a treatment plant in the Bordeaux area, 97 to 98% COD reductions were achieved using a two-stage, high and low-rate activated sludge process (Racault *et al.*, 1998). The hydraulic retention time (HRT) and sludge concentration varied between 10 and 15 d and 7 000 and 10 000 mg $COD.L^{-1}$, respectively. Forgeat & Ehlinger (1998) also evaluated two operating activated sludge systems. Maximum COD reductions of 95% (21 000 to less than 950 $mg.L^{-1}$), and 97% (27 000 to less than 800 $mg.L^{-1}$), were observed. Once again, the final COD levels did not reach legal limits and a further polishing step would be needed.

Another type of activated sludge treatment process, the jet loop reactor, relies on high O_2 transfer efficiencies of 0.5 to 4 kg $O_2.kWh^{-1}$. This system was shown to be suitable for the treatment of brewery wastewater where a COD reduction of 97% was achieved for a period of 5 weeks at an organic loading rate (OLR) of 50 kg $COD.m^{-3}.d^{-1}$ (Bloor *et al.*, 1995). Although settleability was satisfactory, some mobile non-flocculating bacteria caused the effluent to be cloudy and this played a role in the high suspended solid concentration of 200 to 350 $mg.L^{-1}$. Further improvement of effluent quality would thus be a necessity before final discharge.

A jet loop reactor of 15 dm^3 was also used for the aerobic treatment of cellar wastewater with a COD of 800 to 12 800 $mg.L^{-1}$ collected from various wineries in Italy at different periods of the year (Petruccioli *et al.* 2002). The OLR varied between 0.4 to 5.9 kg $COD.m^{-3}d^{-1}$ and the HRT varied between 2.1 and 4.4 d. At an OLR of 1.4 to 4.0 kg $COD.m^{-3}d^{-1}$, the COD removal efficiencies were between 96 and 98%. Even at a loading rate of 5.9 kg $COD.m^{-3}d^{-1}$ the COD

reduction was still above 90%. However, legal COD limits for discharge were once again not met.

Sequencing Batch Reactors – Sequencing batch reactors (SBRs) rely on the same principles for aeration and sedimentation as the ASS process. The major difference is that only one tank is used in which both processes take place. The process works in five steps: fill; react (aeration); sedimentation/clarification; decant; and idle. A portion of the settled sludge is retained in the reactor between runs so as to sustain an active biological community. It is, however, important that there is cell wastage between runs in order to maintain a nominally steady-state microbial population (Tchobanoglous & Burton, 1991; Nazaroff & Alvarez-Cohen, 2001). .

Sequencing batch reactors can also function anaerobically (Nazaroff & Alvarez-Cohen, 2001). To promote this condition, alternate electron acceptors are added in the place of the aeration step. If so desired, SBRs can be operated as sequential anaerobic/aerobic modes to promote a better degradation phase under both conditions.

Houbron *et al.* (1998) evaluated the performance of an aerobic SBR for a moderately sized winery in Narbonne from 1994 to 1996. The inlet COD loading varied between 2 000 and 5 000 $mg.L^{-1}$. The COD was reduced by 93 to 96% and the treated wastewater had a residual COD of 240 to 280 $mg.L^{-1}$. Total Kjeldahl nitrogen (TKN) was also reduced by 50%. Once again legal standards for COD was not met.

Andreottola *et al.* (2002) examined the application of the sequencing batch biofilm reactor (SBBR) for cellar wastewater treatment on pilot-scale. This SBBR combined the advantages of the SBR, such as minimal space requirements and possibility of cycle modifications during plant operation, and the advantages of fixed biomass systems, such as treatment of high organic loads. The COD removal efficiency was between 85 and 99% at organic loads up to 8.8 kg $COD.m^{-3}d^{-1}$.

Trickling filters – Trickling filters (TFs) have been used since 1893 (Tchobanoglous & Burton, 1991). As in the ASS process, TFs rely on wastewater degradation when the organic material is brought into contact with high

concentrations of microorganisms in the presence of O_2. Air is provided through ventilation ports at the bottom of the reactor and moves along the pores through the filter media (Nazaroff & Alvarez-Cohen, 2001). Aeration can be done mechanically or more often is achieved by exposing surface areas of the wastewater to the atmosphere. Air moves as a result of natural convection caused by temperature differences between ambient air and the air inside the filter (Green & Kramer, 1979; Nazaroff & Alvarez-Cohen, 2001).

A TF does not, as the name suggests, separate solids from wastewater in a physical manner. It rather reduces BOD and SS by oxidation (Green & Kramer, 1979). The filter usually consists of a circular or rectangular tank, which is filled with inert and highly permeable material such as stone, plastic or wood on which growth of microorganisms and subsequent biofilm formation are promoted (Tchobanoglous & Burton, 1991; Bitton, 1999; Nazaroff & Alvarez-Cohen, 2001).

The biological community inside the filter includes aerobic, facultative and anaerobic bacteria, fungi, algae and protozoans (Tchobanoglous & Burton, 1991). The most active bacterial genera are *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Alcaligenes*, *Sphaerotilus*, *Nitrosomonas* and *Nitrobacter*. The algae e.g. *Euglena* and *Chlorella*, are important as they produce O_2 needed within the filter during the daytime (Bitton, 1999). Other forms of bio-life such as worms, insect larvae and snails, are also present (Tchobanoglous & Burton, 1991; Bitton, 1999).

The wastewater is distributed through nozzles attached to arms that rotate horizontally round the central vertical axis of the filter. The wastewater is allowed to trickle to the bottom of the reactor where it is recollected (Nazaroff & Alvarez-Cohen, 2001). It is necessary to clarify this effluent as it may contain biomass from the reactor (Green & Kramer, 1979). Sedimentation in settling tanks (humus tanks) is often the answer to separate solids from the treated wastewater (Tchobanoglous & Burton, 1991).

It is important to note that the environmental conditions differ considerably within the filter (Nazaroff & Alvarez-Cohen, 2001). The organisms at the bottom of the reactor are exposed to wastewater with a lower BOD than those at the top. At a specific height within the reactor, the organisms on the outside of the biofilm are exposed to a high BOD and dissolved O_2 level while those attached to the surface material may not get sufficient O_2 and nutrients. This explains the formation of an

anaerobic environment near the material surfaces (Tchobanoglous & Burton, 1991).

When growth of the biomass is too rapid, the pores through which wastewater should flow get clogged and the wastewater forms pools at the top of the filter bed (Nazaroff & Alvarez-Cohen, 2001). This phenomenon is usually referred to as "ponding" and is often the reason for a trickling filter not functioning optimally. Ponding frequently occurs when treating meat-processing wastewater as the high protein content of these waters produce heavy biological growth (Gilde & Aly, 1976).

Trickling filters involve relatively high initial capital costs, but they are usually lower than those for ASSs (Green & Kramer, 1979). More space is required than for ASSs, but still far less than for lagoon systems. Of the major advantages is that the TF needs little operator attention; BOD and SS removal efficiency and nitrification are generally good; and they can be used in series with other trickling filters or other biological oxidation systems.

Trickling filters do, however, have certain drawbacks: the operator does not have much control over the process; filter sludge may break loose and cause clogging; start-up time is usually three to four weeks (not favourable for seasonal food and beverage processing operations); organic, hydraulic or pH shock loads or difference in temperature can upset the system; and standard and intermediate-rate filters often have odour problems and may lead to the breeding of flies (Green & Kramer, 1979).

This system has been shown to be feasible for the treatment of cellar wastewaters. Roux *et al.* (1998) developed a full-scale aerobic filter using a submerged bacterial blanket and aerated the system with a microbubble diffuser. When this was combined with a clarifier for sludge recovery it resulted in a 96.8% COD reduction of wastewater of an initial COD of 4 000 $mg.L^{-1}$. Again, COD was not efficiently removed to reach legal limits.

Rotating biological contactors – The rotating biological contactor (RBC) process was first used for the treatment of municipal wastewater in Europe (Green & Kramer, 1979). The RBC consists of circular disks of polystyrene or polyvinyl chloride that are mounted horizontally on a shaft (Tchobanoglous & Burton, 1991; Bitton, 1999). The disks are about 40% submerged in wastewater and the shaft

turns slowly to bring different parts of the disks into contact with the wastewater. Microorganisms attach to the disks and assimilate nutrients from the wastewater and form 1 to 4 mm thick biofilms (Green & Kramer, 1979; Bitton, 1999). These biofilm organisms that include Eubacteria, filamentous bacteria, protozoa and metazoa are mainly responsible for the BOD removal (Bitton, 1999).

The rotation of the disks has several functions (Tchobanoglous & Burton, 1991). It promotes O_2 transfer while removing excess solids from the disks by force shearing.

Although the RBC has moderately high capital costs, including housing costs to protect against weather damage, it has low operational and maintenance costs. It has low power requirements for aeration and does not need much space or operator attention. RBCs can withstand organic shock loads and are of great importance for seasonal food and beverage processors as they only take 1 to 2 weeks for start-up. The BOD, SS and ammonia nitrogen can be sufficiently removed when the HRT is not too high. It generally also does not lead to fly or odour problems (Green & Kramer, 1979).

A study in Singapore indicated that even under high ambient temperatures, RBCs could yield removal efficiencies of 90 to 94% for loading of 1 to 21 g TOC.$m^{-3}d^{-1}$ (Wilson & Lee, 1997). In this case the pH of the wastewater was kept between 6.0 and 7.7 by the addition of sodium bicarbonate. The wastewater consisted of a mixture of peptone, glucose, meat extract and several nutrients normally present in most wastewaters. It was found that overloading produced a greyish to black biofilm and the reactor bulk liquid became anaerobic. This, however, did not affect the RBCs performance and the possibility of a reactor functioning as a facultative process was also considered.

Lagoon technology – The function of a lagoon is to provide a holding basin for solids to settle and to degrade organic material by biological oxidation processes. Oxidation is achieved by microscopic organisms that naturally occur within the lagoons (Green & Kramer, 1979).

The original lagoons were merely used as holding ponds for wastewater at sites convenient for the processor. With time the physical and biological reactions within the lagoons were better understood and different types of lagoons evolved (Gilde & Aly, 1976). Storage lagoons are usually used for food processors where

large volumes of seasonal wastewaters are produced. Before entering into the lagoon, the wastewater is screened to remove gross solids and some suspended solids. During storage, anaerobic breakdown of organic material and sedimentation of suspended solids take place. After 90 to 120 d the partially treated effluent is discharged during high river flows. The organic reduction is often very low and this treatment option on its own is not enough to reach the levels specified by law. Disposal of the wastewater is regulated to ensure that there is no considerable change in the BOD or reduction in dissolved O_2 in the receiving stream. Although the season's wastewater can be stored at a low initial cost and minimal sludge handling problems, this method has the disadvantage of severe odour problems.

Oxidation ponds or facultative ponds are shallow (1 to 2.5 m in depth) lagoons typically having aerobic and anaerobic zones (Bitton, 1999). Oxygen for aerobic degradation is provided by algal photosynthesis and can be maintained by recirculation or aeration (Green & Kramer, 1979). The algae use the CO_2 produced during oxidation as carbon source (Tchobanoglous & Burton, 1991).

In these ponds, settled sludge is stabilised anaerobically (Gilde & Aly, 1976) resulting in the formation of CO_2, H_2S and methane (CH_4) in the absence of O_2 (Tchobanoglous & Burton, 1991). These gases are either oxidised by the aerobic bacteria or vented to the atmosphere. Between the two layers, a layer functioning both aerobically and anaerobically is found. Here degradation is carried out by facultative bacteria (Green & Kramer, 1979; Tchobanoglous & Burton, 1991).

To minimise odours and prevent mosquitoes from breeding, which often occur in oxidation ponds, the aerobic and anaerobic zones should be well balanced. Although this type of lagoon has a low initial cost and is easy to operate, degradation is often not enough to reach set legal standards (Gilde & Aly, 1976; Bitton, 1999).

It has been shown that facultative pond systems can also be used to successfully degrade dairy wastewaters (Kilani, 1992). When yoghurt waste was treated in a laboratory-scale facultative stabilisation pond, COD removals of above 70% and soluble COD removals of above 80% were achieved. The sludge detention time was only 7.9 d and the organic loading rate as high as 450 kg $COD.ha^{-1}.d^{-1}$.

Oxygen is provided mechanically or by a diffused aeration unit in aerated lagoons. It differs from other lagoons in the high degree of mixing provided to ensure that the solids stay suspended. This treatment option does not only have low operating costs but has a stable solid retention at low loadings and a high buffering capacity (Gilde & Aly, 1976). Aerated lagoon systems have been shown to be a feasible method to treat food-processing wastewaters: wastewater from a beetroot treatment plant with a BOD of 4 236 $mg.L^{-1}$ could be reduced by 98% after a detention time of 1.1 d (Gilde & Aly, 1976).

Maturation lagoons have also been used to treat wastewaters that had already been treated with other systems such as ASSs or TFs (Green & Kramer, 1979). These maturation lagoons had depths of 0.3 to 1.5 m, and O_2 was supplied by natural aeration, photosynthesis or by mechanical aeration. To ensure aerobic conditions, organic loading was kept low and a solids detention time of 18 to 20 d was the minimum for complete endogenous respiration of residual solids (Tchobanoglous & Burton, 1991; Bitton, 1999).

In anaerobic lagoons, anaerobic microbial communities are used to degrade organic material to CO_2 and CH_4 (Gilde & Aly, 1976). The system relies on heavy organic loads to create the desired anaerobic conditions throughout 2.5 to 9.0 m deep ponds. The BODs of wastewaters are normally reduced by 50 to 80% and a secondary aerobic treatment usually follows for further degradation (Green & Kramer, 1979; Bitton, 1999).

The main disadvantages of anaerobic digestion in lagoon systems are the production of odorous compounds, especially H_2S to which the anaerobic microbes are sensitive. Anaerobic digestion also requires fairly high temperatures and when the temperature drops to below 10° to 15°C, degradation stops (Bitton, 1999; Pescod, 1996). Any further BOD removal can be ascribed to the sedimentation of biologically degradable settleable solids (Pescod, 1996).

Anaerobic ponds have the advantage over facultative ponds of a factor of 1 to 10 or even more in terms of land use (Pescod, 1996). Being high in organic and solid concentrations, waste from meat packaging facilities can be successfully treated by anaerobic lagoons. In this case, oils in the water form a layer on top of the lagoon subsequently stabilizing temperature and anaerobic conditions (Gilde & Aly, 1976; Bitton, 1999).

In 1998 Torrijos & Moletta evaluated the performance of an anaerobic pond in the treatment of cellar wastewater with an average COD of 18 000 $mg.L^{-1}$. The 330 m^3 pond was covered and suspended fishing nets were used for microbial attachment. Chemical oxygen demand removals of 95.3% were achieved.

Natural treatment systems – Natural treatment systems rely on the principle of soil acting as a filter in which microorganisms and plants use the wastewater nutrients to form cellular matter or oxidise compounds to CO_2, H_2O, N_2 and other gases. The most predominant problems with land treatment in general are: land availability; groundwater contamination; interference with soil mechanics by specific minerals; and the plants' increased uptake of compounds that may be toxic to higher life (Green & Kramer, 1979). This technology has been used successfully for the treatment of various food processing wastewaters including canning, diary and poultry processing wastes (Gilde & Aly, 1976) and cellar and distillery wastewaters (Radford, 2002).

Land application

Land application can be subdivided into: overland flow; infiltration percolation; and slow rate irrigation. Land with poor percolating abilities such as clay soils covered with a crop like grass is usually used for overland flow treatment. The function of the grass is to provide a habitat for necessary microorganisms, to prevent soil erosion and to act as a physical filter by retarding water flow over the land (Gilde & Aly, 1976; Green & Kramer, 1979). The land normally should have a slope of 2 to 6% and pre-treated wastewater is discharged in a controlled manner, generally by sprinkle-irrigation. Large concentrations of dissolved and suspended solids are trapped in the soil or cover crop and the wastewater is then recollected for further treatment if deemed necessary (Green & Kramer, 1979).

In contrast with overland flow treatment, infiltration percolation relies on sand acting as a filter (Green & Kramer, 1979). Wastewater is applied at a high rate by sprinklers or by the rapid flooding of basins for a few days. This is then ceased to allow the wastewater to percolate through the sand while microorganisms and plants assimilate the nutrients. The purified effluent recharges the groundwater. The sand is usually covered with water-tolerant

grasses, which are planted to prevent erosion. In this case it is important to remove most of the suspended solids before filtering as the solids may cause clogging of the sand.

Slow rate irrigation involves the application of wastewater to land areas where crops are planted that utilise nutrients and remove water by evapotranspiration. Microorganisms in the soil play a role in the biodegradation of the organic matter. The plant roots then absorb the small or soluble organic compounds as well as the remaining minerals present in the wastewater or formed by microbial degradation (Green & Kramer, 1979). The remaining inorganic compounds are removed from the water through physical adsorption on the soil colloids (Gilde & Aly, 1976). It is recommended that the application rate should not be too high as infiltration is fundamental and a run-off should normally not be allowed (Green & Kramer, 1979).

Land application is an attractive treatment method for wineries that have available land if they comply with local law. Land application also has the additional advantage that crops for grazing can be developed as for example, kikuyu grass is suitable for cellar wastewater irrigation (Water Research Commission, 1993). However, section 21 of the National Water Act, 1998 (Act 36 of 1998) specifically specifies that such water uses are to be controlled. The disposal of cellar wastewater is dealt with in section 21(e): "engaging in a controlled activity as defined in the terms of section 37(i) of the act, namely the irrigation of any land with waste or water containing waste from an industrial activity (Republic of South Africa, 1998)". For irrigation, legislation requires: "the electrical conductivity to be below 200 $mS.m^{-1}$; pH to be between 6.0 and 9.0; the faecal coliforms to be below 100 000 per 100 mL; and the sodium absorption ratio to be below 5". Furthermore, to irrigate less than 50 $m^3.d^{-1}$ the COD should be below 5 000 $mg.L^{-1}$. If less than 500 $m^3.d^{-1}$ is to be irrigated, the required COD drops to 400 $mg.L^{-1}$ (Anon., 1999).

The environmental impact of land application has been studied in Germany. Heil & Müller (1998) evaluated the application of cellar wastewaters at rates of less than 5 mm or 50 $m^3.ha^{-1}$. They found that the soil fertility did not improve significantly and the accumulation of heavy metals and other toxicants were undetectable. Detergents used in the wine industry could, however, be detected.

The use of biodegradable lyes, acids, peroxides and alcohols for cleaning operations were thus rather recommended.

In contrast, environmental evaluations in South Africa showed that three out of 10 wineries using land application were found to pollute the soil specifically, where the organic component leached through the soil to the water table (Mulidzi *et al.,* 2002). It was found that the three polluting wineries disposed of their wastewaters onto sandy soils. The sand at the upper extremity of the watertable became black and anaerobic decomposition took place in these sections resulting in strong odours when disturbed. At one winery, the drainage carried organic material from the wastewater directly into a river.

Wetlands

Wetlands can be described as submerged land areas covered with surface vegetation capable of survival in watery conditions with depths of less than 0.6 m (Tchobanoglous & Burton, 1991). The plants have several functions as they: provide surface areas for bacterial film attachment; act as a filter to separate wastewater constituents from the actual wastewater; transfer O_2 into the water; and prevent the growth of algae as they limit sunlight reaching the water. The plants and microorganisms are responsible for the removal of BOD and nutrients such as N and P (Bitton, 1999). Both natural and constructed wetlands have been used for wastewater treatment (Tchobanoglous & Burton, 1991).

A wetland constructed as part of a waste management system for dairy parlour wastes was monitored over a two-year period (Geary & Moore, 1999). It consisted of two wetland trenches with respective surface areas of 127 and 168 m^2 each, with a total volume of 100 m^3. The average of the mass loading rates onto the wetland were 5.6 $g.m^{-2}.d^{-1}$ for BOD, 2.6 $g.m^{-2}.d^{-1}$ for organic nitrogen, 3.2 $g.m^{-2}.d^{-1}$ for ammonia, and 1.5 $g.m^{-2}.d^{-1}$ for total phosphorous (TP). The detention times varied between 9 and 21 d. The mean monthly pollutant reductions were calculated and found to be 61% for BOD, 43% for organic nitrogen, 26% for NH_3 and 28% for TP. At high loadings, pollutants appeared to accumulate in the wetland, as was the case when the wetland became saturated with phosphorous. It was concluded this wetland was too small to be used to treat this high strength dairy waste over a longer term.

Shepherd *et al.* (2001) examined the performance of a pilot-scale sub-surface flow constructed wetland combined with an upflow sand pre-filter. The wetland was 6.1 m long, 2.4 m wide and 1.2 m deep, and was used to treat cellar wastewater from a moderate sized winery (2 million cases per year). The wastewater had a COD ranging from a minimum of 600 $mg.L^{-1}$, collected during the night, to a maximum of 45 000 $mg.L^{-1}$, collected during the day. The flow rate was 80 to 170 $m^3.d^{-1}$. A COD reduction of 98% and total suspended solid removal of 97%, were achieved. At high organic loading rates (1 640 kg $COD.ha^{-1}d^{-1}$), the wetland did not exhibit negative responses except for reduced plant growth at the front end of the system.

In a separate study it was reported that the Boars Rock wine cellar in Australia pressed 6 000 tons of grapes annually and used a constructed artificial wetland for the treatment of its cellar wastewater (Van Schoor, 2002). The 300 m long wetland had a total area of approximately 1.5 ha and consisted of a combination of coarse (50 mm diameter) and fine (20 mm diameter) gravel and reeds. The wetland was sealed with a layer of clay. After solid particle removal by using basket sieves and gravitation, the wastewater moved through the complete length in 12 to 20 d ending in an irrigation dam. The system increased pH from 3.8 to 7.4 and led to reduced BOD from 11 600 to 8 $mg.L^{-1}$ in the peak harvest period. (Wonderful – but can this be true?)

The effectiveness of constructed wetlands may vary according to the region and the type and volume of wastewater to be treated (Van Schoor, 2002). In South Africa, three small-scale experimental wetlands (6 m x 2.4 m x 1 m) and two large-scale wetlands (45 m x 4 m) have been constructed at the Spier and Goudini wine cellars, respectively. These are used to investigate the type of construction material, layout, type of vegetation, seasonal variability of wastewater, amount and composition of wastewater generated and the size of the wetland required to treat specific wastewater volumetric flows.

Anaerobic digestion – The primary goal of anaerobic digestion is to degrade organic material present in wastewater to CH_4 and CO_2 in the absence of molecular O_2. In this way it reduces the organic impact that polluted water will have on the environment. The biogas can be collected and used as energy source (Puñal & Lema, 1999; Nazaroff & Alvarez-Cohen, 2001).

Anaerobic digestion has been applied in the treatment of household wastewater in septic tanks, slurries in digesters and sewage sludge in municipal treatment plants since the end of the nineteenth century (Van Lier *et. al.*, 2001). Since then the growing food industry and international oil crisis stimulated a rapid renewal in reactor design and today high rate anaerobic digesters are used for the treatment of various wastewater types including many industrial and municipal wastewaters (Hickey *et al.*, 1991; Verstraete & Vandevivere, 1999; Van Lier *et al.*, 2001). Several anaerobic configurations, that efficiently separate the solids independently from the hydraulic retention times, are used in practice. This allows for the accumulation of high biomass concentrations at relatively low hydraulic retention times (Hickey *et al.*, 1991).

Microbial metabolism

The biomass of anaerobic digesters seldom contains fungi and protozoa and is mostly dominated by bacteria (Bitton, 1999). Four major bacterial categories function in a synergistic relationship and are responsible for anaerobic organic compound degradation as is summarised in Fig. 1. The four major groups are the hydrolytic, fermentative acidogenic, acetogenic and the methanogenic bacteria (Bitton, 1999). The different population groups of bacteria are dependent on each other for the supply of the nutrients they require and the maintenance of an appropriate environment (Ditchfield, 1986).

The facultative and strict anaerobes, especially syntrophic acetogens and methanogens, are able to grow under conditions of extremely low free energy availability. Some of these organisms can even reverse catabolic reactions in order to gain an energy advantage (Hickey *et al.*, 1991).

The hydrolytic bacteria are responsible for the hydrolysis of complex organic molecules such as proteins, cellulose, lignin and lipids, into soluble monomer molecules such as amino acids, glucose, fatty acids and glycerol. Extracellular enzymes like cellulases, proteases and lipases catalyse the process (Tchobanoglous & Burton, 1991; Schmidt & Ahring, 1996; Bitton, 1999). Acidogenic bacteria convert the products of the hydrolysis step to organic acids, alcohols, ketones, acetate, CO_2 and hydrogen (H_2). The precise products vary according to the wastewater composition, type of bacteria and growth conditions

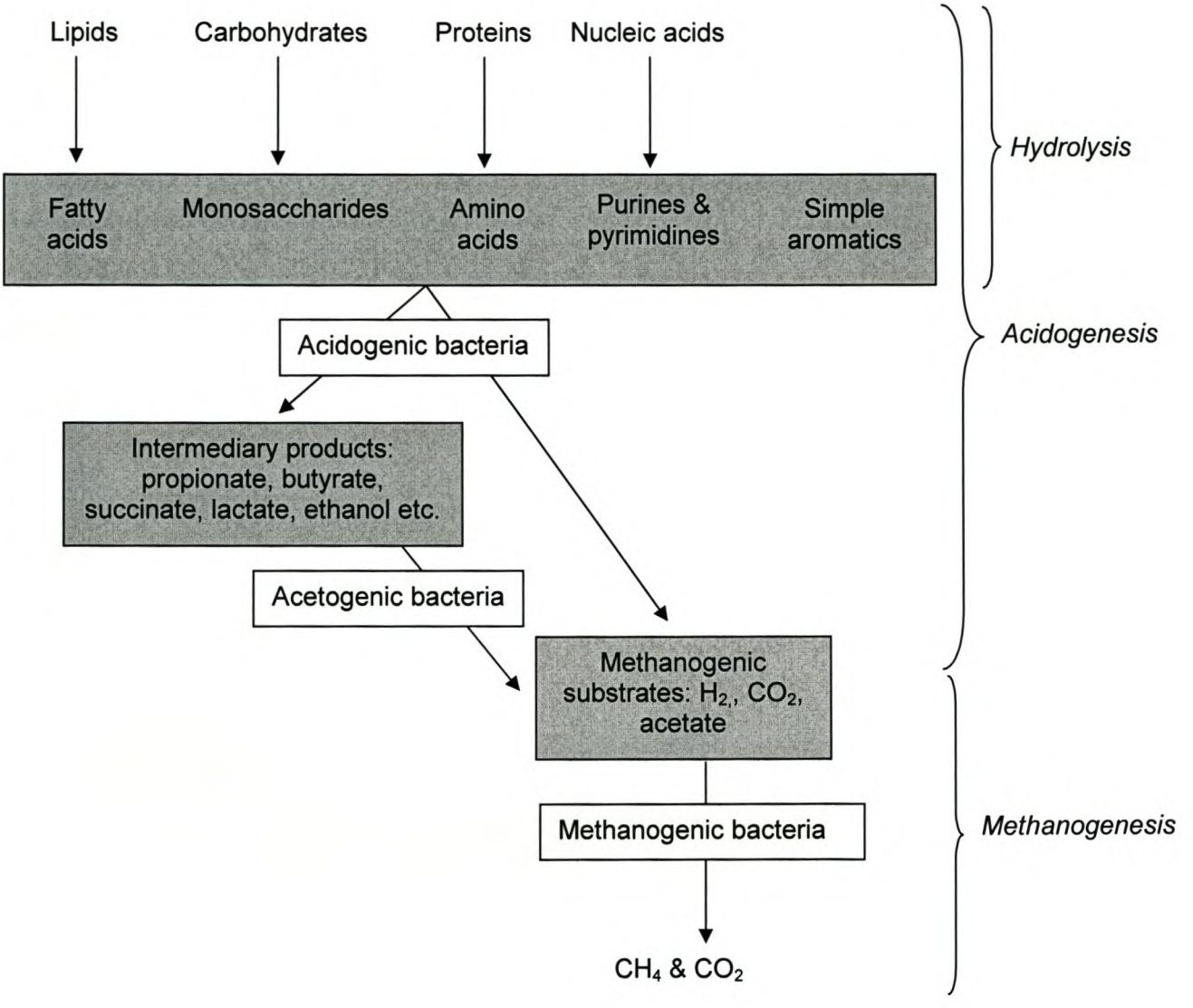


Figure 1. Schematic diagram of the carbon flow in anaerobic digesters (adapted from Tchobanoglous & Burton, 1991).

present, such as temperature and pH (Iza *et al.*, 1991; Bitton, 1999). The acetogenic bacteria convert fatty acids such as propionic and butyric acids and alcohols into acetate, H_2 and CO_2. A low H_2 pressure is necessary for the conversion to take place (Ditchfield, 1986; Schmidt & Ahring, 1996; Bitton, 1999). The H_2 pressure should be lower than 3.3×10^{-5}, 3.2×10^{-4} and 1.8×10^{-1} atm for propionate, butyrate and ethanol, respectively, to be converted to acetate (Mata-Alvarez, 2003). A relatively high H_2 partial pressure can be prevented by a symbiotic relationship between acetogenic bacteria and the methanogens as the methanogens help achieve the low hydrogen pressure required by the acetogenic bacteria (Tchobanoglous & Burton, 1991; Bitton, 1999).

The methanogenic group is composed of both Gram-positive and negative bacteria of varying morphologies (Bitton, 1999). They grow slowly in wastewater with generation times of 3 d at 35°C and 50 d at 10°C. For purposes of anaerobic digestion, the methanogens can generally be divided into two major metabolic groups namely, hydrogenotrophic and acetotrophic methanogens. The hydrogenotrophic methanogens convert H_2 and CO_2 into CH_4 using an active hydrogenase (Bitton, 1999):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \Delta G = -135.6 \text{ kJ (Mata-Alvarez, 2003).}$$

As can be derived from the above reaction, this group help to maintain the low H_2 partial pressure required for efficient conversion of volatile fatty acids and alcohols to acetate (Bitton, 1999).

The acetotrophic methanogens are responsible for the formation of CH_4 through a decarboxylation process:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \Delta G = -31.0 \text{ kJ (Mata-Alvarez, 2003).}$$

The above reaction accounts for approximately 70% of the CH_4 formed during anaerobic digestion (Ditchfield, 1986; Shieh & Nguyen, 1997).

Each of the four major microbial groups has its own complex nutritional requirements (Hickey *et al.*, 1991). The species dominance and diversity is thus influenced by the wastewater composition. It has been shown that these groups,

especially the acidogenic population, influence the efficiency of the anaerobic digestion process (Britz *et al.*, 1994).

Factors affecting anaerobic digestion

In order for an anaerobic digester to function optimally, the methanogens and other groups of bacteria must be in a state of equilibrium (Tchobanoglous & Burton, 1991). Several factors play a role in achieving the above.

Temperature – Although CH_4 production has been documented at temperatures ranging from 0° to 97°C (Bitton, 1999), the optimum temperatures for the maintenance and optimal functioning are in the mesophilic (30° to 38°C) and thermophilic (49° to 57°C) ranges (Tchobanoglous & Burton, 1991). It is specifically the slow growing methanogens that are sensitive to small deviations from the optimum temperature (Bitton, 1999).

Retention time – The hydraulic retention time must be long enough for adequate metabolism by anaerobic bacteria within the reactor. The precise time, which can reach HRTs of a few hours, is dependent on the: type of reactor, whether the bacteria are attached or not, the wastewater characteristics and environmental conditions (Bitton, 1999).

pH – The pH of the wastewater substrate should be in a starting range of 6.6 to 7.6 (Tchobanoglous & Burton, 1991) as the products of acidogenesis and acetogenesis lower the pH in the reactor content. If the pH drops below 6.5, methanogenesis is slowed and if the pH decreases further, the process can be terminated (Nazaroff & Alvarez-Cohen, 2001) leading to complete reactor failure. Sufficient alkalinity (1 000 to 5 000 $mg.L^{-1}$) is always necessary for the digestion to proceed satisfactorily and serve as a buffer to prevent a sudden decrease in pH, which would lead to reactor failure (Tchobanoglous & Burton, 1991).

Composition of wastewaters – The microbial consortium present in anaerobic digesters can produce CH_4 from carbohydrates, proteins, lipids and complex aromatic compounds but compounds such as lignin and *n*-paraffins are more difficult to degrade (Bitton, 1999). When the organic loading of degradable substrates is suddenly increased considerably, the hydrolytic and acidogenic bacteria, as a result of their rapid growth abilities, will metabolise the additional easily degradable compounds, with the subsequent increase in volatile fatty acids. The methanogens that are slower to react to changes and have longer growth

rates are not able to degrade the increased acid concentration and the pH of the system will decrease. This lower pH will further inhibit methanogenesis, as H_2 will not be removed from the system (Ditchfield, 1986). Under these environmental conditions reactor acidification and subsequent failure will result.

The wastewater should have sufficient amounts of nutrients, such as nitrogen and phosphorous for optimum performance. Souza (1986) suggested COD/N and COD/P ratios equal to or below 70 and 350 respectively, to be adequate.

Trace elements, including iron, cobalt, molybdenum and nickel, are essential for the optimum efficiency of the anaerobic digestion system (Bitton, 1999). Nel *et al.* (1985) used a downflow fixed film reactor treating a petrochemical wastewater to investigate the addition of trace elements during anaerobic digestion. They found that the addition of silicon, selenium, tungsten and nickel improved the reactor performance. This was ascribed to the trace elements being beneficial to the methanogenic population.

Various anaerobic systems, especially the upflow anaerobic sludge blanket (UASB) system, rely on the formation of bacterial agglomerates or granules for successful operation. Granulation does help to retain the microbial population in the reactor (Schmidt & Ahring, 1996). It has been shown that calcium concentrations between 150 and 300 $mg.L^{-1}$ could enhance granulation (Yu *et al.*, 2001). Goodwin *et al.* (1990) examined the effect of nutritional deficiencies on initial performance and granulation in the UASB reactor. They reported that a phosphate deficiency inhibited the process and deficiencies in magnesium and calcium or in trace elements ($FeSO_4$, $NiSO_4$, $MnSO_4$, $ZnSO_4$, H_3BO_3, $CoCl_2$, $CuSo_4$, $H_3PO_4 \cdot 12MoO_3$) caused possible methanogenic upset indicated by high VFA concentrations.

Competition – At a COD/SO_4 ratio of 1.7 to 2.7 methanogens and sulphate-reducing bacteria compete for acetate and H_2. An increase in the ratio favours methanogenesis while a decrease favours the sulphate reducers (Bitton, 1999). Sulphate reduction may lead to diminished CH_4 production (Shieh & Nguyen, 1997).

Toxicants – Various compounds are considered to be toxic to anaerobic bacteria, especially to the methanogens. As methanogens are obligate anaerobes, trace levels of O_2 can be detrimental (Tchobanoglous & Burton, 1991).

Other toxicants are ammonia, chlorinated hydrocarbons, formaldehyde, long-chain fatty acids, cyanide, sulphide, tannins and high salinity concentrations (Bitton, 1999). It is thus important to continuously monitor the levels of toxicants present in substrate fed during the anaerobic digestion process. Control parameters, which can be utilised to measure progress and indicate system disruption within the reactor, include volatile fatty acids, alkalinity, pH, biogas production rate, CO_2 content of biogas, volatile solids reduction, temperature, volatile solids loading and sensory assessments (Ross *et al.*, 1992).

Anaerobic processes

The various anaerobic treatment options are all based on the ability to retain the bacterial biomass within the reactor and to prevent biomass washout (Borja *et al.*, 1993). The bacteria may be suspended or attached.

Attached-growth treatment processes – two common anaerobic treatment processes are the anaerobic filter and the expanded-bed systems (Bitton, 1999). The anaerobic filter can be seen as the anaerobic equivalent of the aerobic trickling filters. The anaerobic filters normally consist of a column filled with a variety of solid materials on which the anaerobic bacteria may grow (Tchobanoglous & Burton, 1991). The wastewater enters the column at the top or bottom of the system and then flows in the opposite direction. As it passes the attached bacterial growth, organic material is utilised (Shieh & Nguyen, 1997).

The expanded-bed in contrast consists of a media bed such as sand, activated carbon or expanded aggregate on which bacterial growth may develop (Tchobanoglous & Burton, 1991). Wastewater flow upward through the media at a flow rate high enough to attain an expanded bed (Bitton, 1999). The wastewater circulates and recycles through the reactor while it is degraded. The reason is to dilute incoming waste and to provide an adequate flow to keep the bed in an expanded condition (Tchobanoglous & Burton, 1991).

One type of expanded bed reactor is the fluidised-bed reactor. Is this case the carrier medium is kept in suspension by the drag forces exerted by the upflowing wastewater. This enlarges the area where biofilm formation and growth will occur (Shieh & Nguyen, 1997). The feasibility of the fluidised bed reactor for the thermophilic treatment of wine distillery wastewater has been investigated

(Perez *et al.*, 2001). A COD reduction of 81.5% at an OLR of 32 kg COD $m^{-3}d^{-1}$ was achieved using a laboratory-scale fluidised bed reactor.

Wine distillery wastewater has also been treated in a down-flow fluidised bed (Garcia-Calderon *et al.* 1998). Down-flow fluidisation involves floatable particles with a specific density smaller than the liquid, being fluidised downward by a concurrent flow of liquid. Perlite particles were used as fluidised material to achieve an 85% total organic carbon (TOC) removal at an OLR of 4.5 $TOC.m^{-3}d^{-1}$.

Suspended-growth treatment processes – Two common suspended-growth treatment processes are the anaerobic contact and upflow anaerobic sludge blanket (UASB) processes. The anaerobic contact process is similar to the activated sludge process, the difference being the formation of anaerobic conditions within the former. The process involves mixing of the substrate wastewater with an anaerobic sludge culture followed by settling and returning of the sludge to the mixing chamber (Bitton, 1999).

In contrast, the basic principle of the UASB involves the upflow of substrate feed passing through a bed of highly activated sludge granules, which convert the organic compounds within the influent to CO_2 and CH_4 (Hickey *et al.*, 1991). The UASB process relies on the spontaneous formation of granular conglomerates of anaerobic bacteria (Van Lier *et al.*, 2001). Granular sludge settles well and this prevents the washout of the anaerobic bacteria (Bitton, 1999).

Chemical Treatments

Chemical treatments are normally used as secondary or tertiary treatment options and utilises different forms of chlorine, O_2, ozone (O_3) and permanganate to oxidise compounds in the wastewater. These reactions are specifically important in the removal of organic solids and are often suited to disinfect and remove colour, taste and odour compounds from effluent that has already undergone primary and secondary treatment (Green & Kramer, 1979; Nazaroff & Alvarez-Cohen, 2001).

E. THE UASB PROCESS

The UASB process, developed in the 1970s by Lettinga and co-workers (Lettinga & Hulshoff Pol, 1991), is today used for the successful treatment of a wide variety of industrial wastewaters throughout the world (Driessen *et al.*, 1994; Schmidt & Ahring, 1996). The basic principle of the UASB involves the upflow of influent passing through a bed of highly activated sludge granules, which convert the organic compounds within the influent wastewater to CO_2 and CH_4 in the absence of O_2 (Hickey *et al.*, 1991). The granules are formed by natural self-immobilization of the biomass where no support material such as Rasching rings or clay, is present (Schmidt & Ahring, 1996).

Working principles of UASB reactor

The UASB reactor, as illustrated in Fig. 2, can be divided into four main components: the sludge bed; sludge blanket; gas-solids separator (GSS); and a settling compartment. The sludge bed is a layer of granular biomass settled at the bottom of the reactor. This is covered by a sludge blanket, which is a mixture of granular sludge and gas formed during the anaerobic breakdown of organic compounds. Wastewater is pumped through the reactor from the bottom and both the sludge bed and sludge blanket are responsible for degradation. The GSS device then facilitates the separation of the formed gas from the liquid and granular mass. The settling compartment is an inactive zone where granular sludge particles can settle back in the reactor. The bacteria that are not already flocculated or dispersed are washed out with the effluent (Lin & Yang, 1991; Schmidt & Ahring, 1996).

Granulation

The bacteria responsible for anaerobic breakdown of wastewater consist of different trophic groups where each group of bacteria plays a specific role in the breakdown of organic matter to CH_4 and CO_2 (Schmidt & Ahring, 1996). Thus, the selection of a suitable inoculum is essential for efficient start-up and operation of the UASB. The inoculum material should contain microorganisms that induce

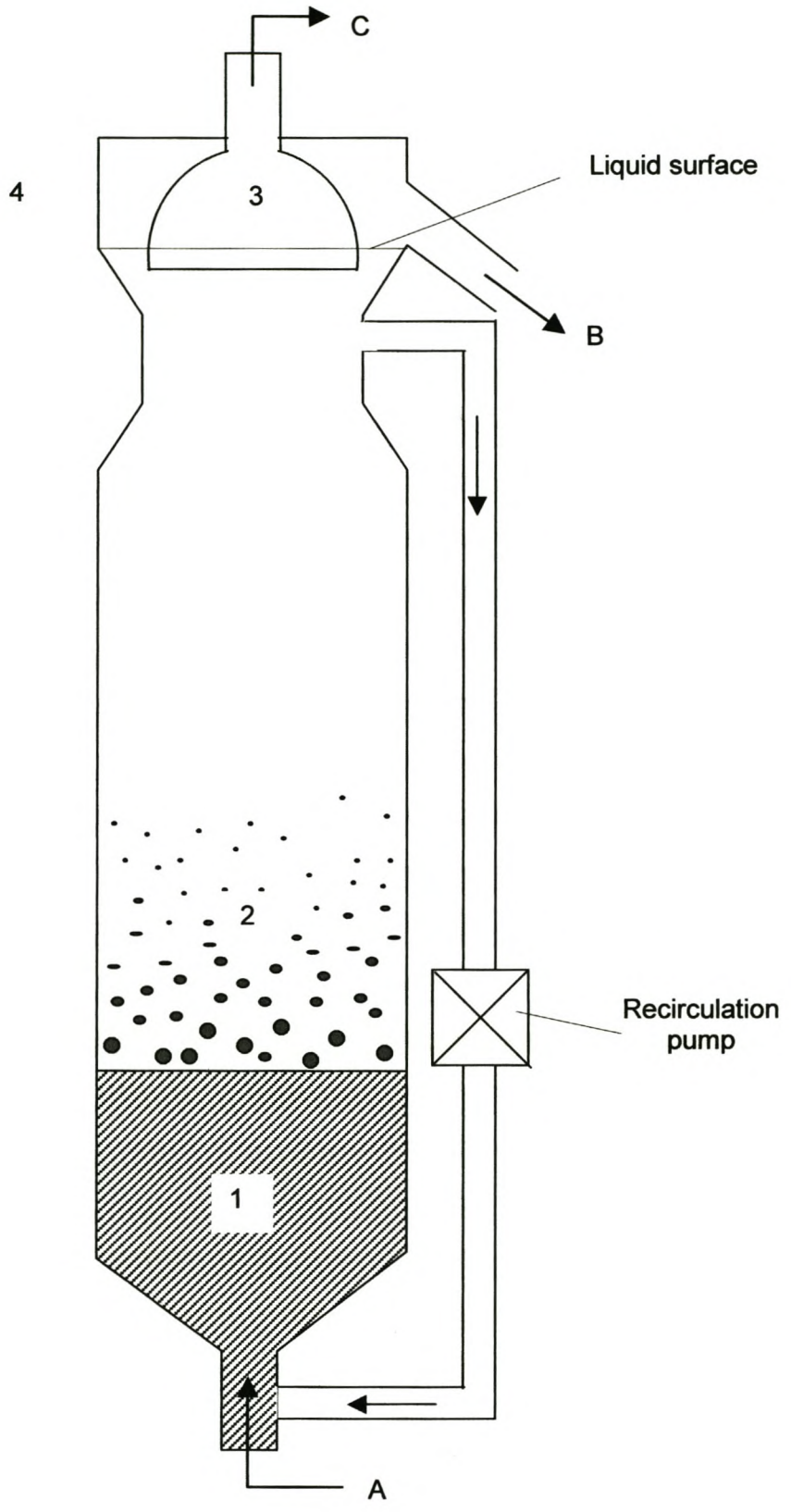


Figure 2. Schematic representation of a UASB reactor: 1 granular sludge bed; 2 sludge blanket; 3 gas/liqiud separator; 4 settling compartment; A influent; B effluent; C biogas outlet (Schimdt & Ahring, 1996).

granule formation and are able to degrade the organic compounds in the specific wastewater (Hickey *et al.*, 1991).

Start-up can be carried out with sludge containing the required anaerobic populations or by using granules from existing reactors treating the same or similar wastewaters. Where the treatment of a specific industrial wastewater is the first of its kind, the start-up time can take up to six months or even longer (Lund, 1988).

The typical shape of the granules is spherical with a diameter varying between 0.14 and 5 mm. The precise diameter is dependent on the wastewater used, the operational conditions and the monitoring analytical method (Schmidt & Ahring, 1996). As the OLR of industrial reactors change, the granules may also change in colour and elemental composition (Kosaric *et al.*, 1990). The inorganic material varies between 10 and 90% of the granule dry mass and the main components of the ash are calcium, iron and potassium. The inorganic compounds appear to be of less importance in the granule structure and maintenance than the extracellular polymers (ECP) in the granular sludge. The extracellular polymers consist mainly of protein and polysaccharides and form 0.6 to 20% (m/m) of the volatile suspended solids (Schmidt & Ahring, 1996).

Very little is known about the mechanism of granule formation and maintenance or disintegration of granules (Kosaric *et al.*, 1990). A possible explanation for granule formation compiled by Schimdt & Ahring (1996) is the formation of ECP as shown in Fig. 3. In their explanation dispersed bacteria are negatively charged and thus repel each other while the extracellular polymers neutralize the charge and thus promote aggregation.

Britz *et al.* (2002) showed that granulation could be enhanced in batch culture units inoculated with raw anaerobic sludge. Since a batch system containing lactate as carbon source showed a 559% increase in granules within 20 d, it was assumed that the lactate medium could create a favourable environment for propionic-acid-producing bacteria. Propionate producers are generally efficient ECP and aggregate formers (Mulder *et al.*, 1989).

It has also been shown that biomass accumulation and granulation may be improved with the introduction of Ca^{2+}. It is believed that the calcium enhances granulation by acting as a link between extracellular polymers and bacterial cells. Calcium could also play a role in multiplication of cells and thus development of granules (Yu *et al.*, 2001).

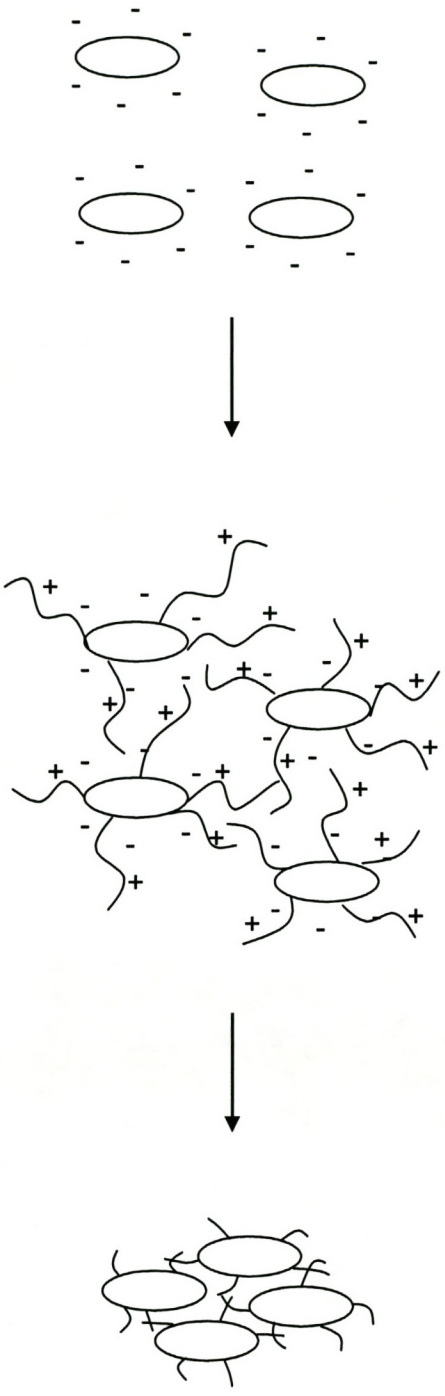


Figure 3. The role of surface charge and production of extracellular polymers in the development of granules (Schmidt & Ahring, 1996).

Anaerobic bacteria present in the granules are aceticlastic methanogens *Methanoseata* spp. (previously *Methanotrix)* and *Methanosarcina* spp., H_2 and formate utilizing methanogens e.g. *Methanobacterium formicicum, Methanobacterium thermoautrophicum* and *Methanobrevibacter* spp. and microcolonies of syntrophic bacteria. The precise internal organization of the various bacteria is dependent on the wastewater composition and the dominating metabolic pathway within the granule. The internal organization is most often beneficial for the breakdown of the wastewater (Schmidt & Ahring, 1996).

Applications

UASB systems have successfully been employed for the treatment of various wastewaters from the food industry. These include maize, meat and dairy processing, brewery and fruit cannery and cellar wastes (Ross, 1989; Strydom *et al.*, 1997; Puñal & Lema, 1999; Ronquest & Britz, 1999; Sigge *et al.*, 2002).

Since wastewaters from distilleries are highly polluted, anaerobic digestion may find a wide application in this industry (Driessen *et al.*, 1994). O'Kennedy (2000) showed that a mesophylic lab-scale UASB reactor could reduce the COD of high strength distillery wastewater by more than 90%. The system was optimised to treat wastewater with a pH of 4.7 at an OLR of 30 $kgCOD.m^{-3}d^{-1}$.

It has also been shown that the UASB can be used to treat a lower strength cellar wastewater. In a study, a COD removal of more than 93% at an OLR of 10.1 $kg\ COD.m^{-3}d^{-1}$, a HRT of 14 h and an influent pH of 5.1 were achieved with a laboratory UASB system seeded with granules from a reactor treating cannery wastewater (Ronquest & Britz, 1999). Under these conditions the final effluent COD was 182 $mg.L^{-1}$.

Müller (1998) also studied the efficiency and capability of an UASB reactor in the treatment of cellar wastewater. Removal efficiencies of more than 90% for BOD and COD were reached at COD loadings of 941 to 13 600 $mg.L^{-1}$. In another system, where an hybrid UASB reactor was combined with an anaerobic filter, a 93% COD removal was achieved using cellar wastewater with a strength of 10 000 mg $COD.L^{-1}$. It was found that the hybrid reactor only needed a 1-week start-up period to get back to its previous functioning character after not being in

use for 4 months (Andreottola *et al.*, 1998). Once again, the legal limits for disposal were not reached, thus an additional polishing step would be required.

F. OZONATION

Biological or chemical treatments alone are not always efficient enough to achieve the required result, whether it is to degrade toxic or refractory compounds in wastewater or maximise mineralization. In many cases the required goals cannot be reached economically with only one treatment option. In these cases combined strategies, where the strength of each process may be utilised optimally, are required. It is important to note that when an oxidation process such as ozonation is used as a pre-treatment in a combined biological process, the biodegradability of the wastewater will only increase until a certain maximum is reached. Further ozonation will lead to a decrease in the biodegradability (Gottschalk *et al.*, 2000).

Background

Ozone is an unstable, blue, diamagnetic gas with a distinctive pungent and stimulative odour that can be smell-detected at concentrations as low as 0.01 $mg.L^{-1}$ (Greenwood & Earnshaw, 1984; Xu, 1999; Shirakura *et al.*, 2001). Since O_3 is toxic, maximum human occupational recommended exposure limits for an 8-hour time-weighed average and a short term (15 min) exposure were established as 0.2 and 0.6 $mg.m^{-3}$, respectively (Anon., 1995).

This triatomic allotrope of O_2 is a versatile gas as it disinfects and oxidises while returning to harmless O_2 (Greenwood & Earnshaw, 1984; Rice, 2001). Ozone is a potent oxidant, 5^{th} in thermodynamic oxidation potential after elemental fluorine, chlorine trifluoride, atomic O_2 and hydroxyl free radicals (Graham, 1997). Because of O_3's high oxidation potential, it has faster reaction kinetics than most other oxidants. Therefore lower concentration or shorter contact times are required to complete an oxidation reaction compared with other oxidising agents (Xu, 1999). The oxidation process, which involves free-radical chains as well as peroxo intermediates, can occur with most substances at 25°C (Cotton *et al.*, 1999).

Generation

Since O_3 is reactive, unstable and has a hazardous nature, it should be prepared at the point of use (Greenwood & Earnshaw, 1984). Various methods (Table 3) for O_3 generation include: electrical discharge; electrochemical; photochemical; radiation chemistry; and thermal methods (Gottschalk *et al.*, 2000).

The electrical discharge method (Fig. 4a) involves the flow of O_2 or dry air in glass tubes separating two electrodes to which an electromotive force of 5 000 to 30 000 V is applied. Ozone is formed in a reaction of ionised oxygen atoms and molecular O_2. Several factors influence the final O_3 yield. These factors include the design of the ozonator, the type of carrier gas (air or O_2), temperature, gas flow rate, applied voltage and pressure and moisture content of the gas (Walter & Weber, 1972; Gottschalk *et al.*, 2000).

In the electrolytic O_3 generator, electrolysis of high-purity water leads to the formation of molecular H_2, O_2 and O_3. As illustrated in Fig. 4b, water, O_2 and O_3 leave the cell on the anode side while H_2 is produced at the cathode side. As in the electric discharge method, the temperature influences the O_3 yield. Efficient cooling is thus a necessity. For effective functioning of the cell, all cell material has to be electrochemically stable. To prevent the clogging of the porous anode and cathode high-purity water should be used (Gottschalk *et al.*, 2000).

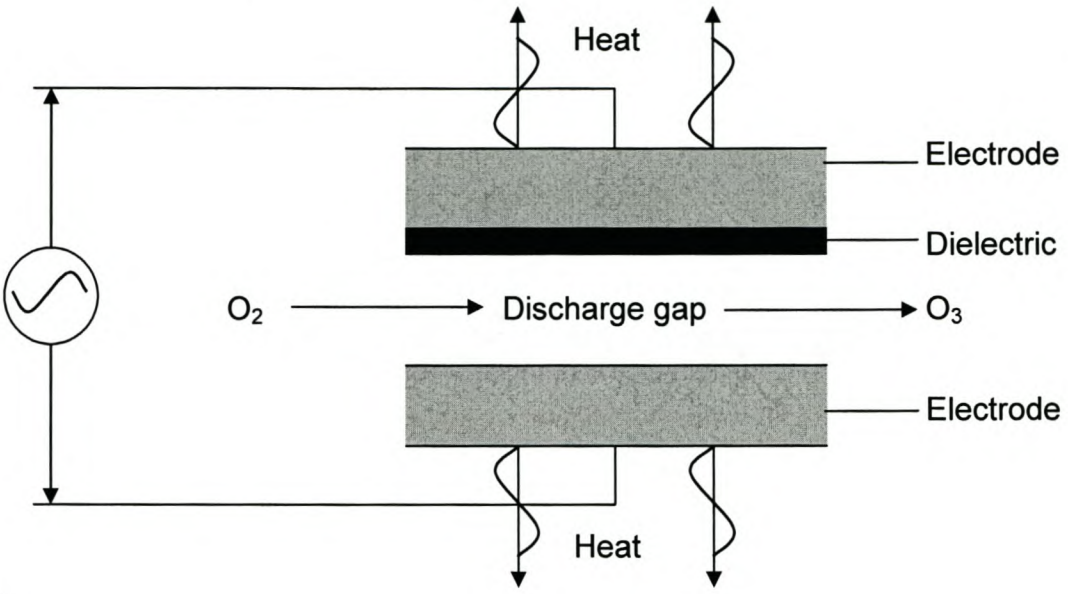
The use of UV light with a wavelength of approximately 185 nm in the generation of O_3 is quite popular when low O_3 concentrations are required. This technology has successfully been used to provide low O_3 concentrations in the atmosphere of cold storage rooms used for eggs and meat (Graham, 1997).

Concentration determination

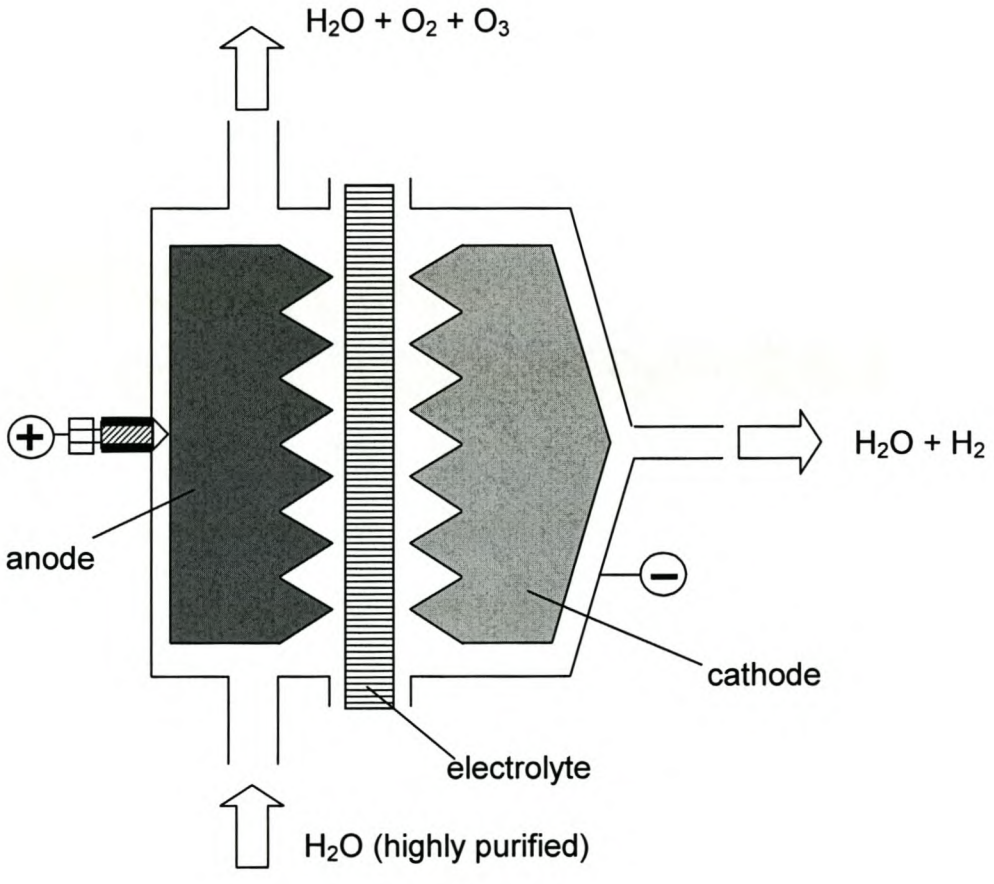
Several methods are available for the determination of O_3 concentrations. The Iodometric Method is used for O_3 in a gas or liquid phase (Gottschalk *et al.*, 2000). This method involves the oxidation of the iodide ion of KI by O_3 to I_2. The I_2 is then instantaneously titrated against $Na_2S_2O_3$ to a pale yellow colour.

Table 3. Summary of methods for O_3 generation (Gottschalk *et al.*, 2000)

Method of generation	**Working Principle**	**Ozone Source**	**Application**
Electrical	Electrical discharge	Air or O_2	Standard
Electrochemical	Electrolysis	Water (highly purified)	Pure water applications – laboratory to small industrial scale
Photochemical	Irradiation	O_2 Water	New technology – laboratory to full-scale
Radiation chemistry	X-rays, radioactive γ-rays	Water (highly purified)	Very seldom – solely experimental
Thermal	Light arc ionisation	Water	Very seldom – solely experimental



(a)



(b)

Figure 4. Ozone Generation: a) Electrical discharge (ASCE & AWWA, 1990); b) Electrochemical (Gottschalk *et al.*, 2000).

Ozone concentration in the gas or liquid phase can also be determined by photometry at the absorption maximum of 254 nm (Gottschalk *et al.*, 2000). The UV intensity is proportional to the O_3 concentration.

In another method the fact that O_3, when in an acidic solution, decolourises indigo trisulphate, is utilised (APHA, 1992; Gottschalk *et al.*, 2000). The decrease in absorbance measured at 600 nm is linear with the increasing O_3 concentration.

The oxidation of iodide yields iodine which in turn can form a radical cation with N,N-diethyl-1,4 phenylenediammonium (DPD). DPD is a red dye and has a stable colour in its radical cation form, with absorption maximums at 510 and 551 nm. The intensity of the dye is proportional to the O_3 concentration. This method is used to measure O_3 when present in liquid form at concentrations as low as 0.02 to 2.5 $mg.l^{-1}$ (Gottschalk *et al.*, 2000).

Another method that is sometimes used when O_3 is present in low concentrations in an aqueous solution is the σ-tolidine/manganese sulphate method (Walter & Weber, 1972). When in acid solution, the O_3 oxidises Mn(II) to Mn(III) that produces a yellow colour when an σ-tolidine reagent is added.

Some reagents, such as benzoflavin, acridine yellow and indigotrisulfate, have a high selectivity to be oxidised by O_3 (Gottschalk *et al.*, 2000). In a reaction with aqueous O_3 it produces light, which can be detected with a photodetector. In this case, the intensity of the light is proportional to the concentration of the O_3.

The membrane O_3 electrode method is useful when O_3 concentration needs to be measured continuously. A gold cathode, silver anode, an electrolyte and a Teflon membrane are required. Ozone is reduced to O_2 at the cathode and silver is oxidised to form Ag^+ and electrons at the anode. This creates an electrical potential that is proportional to the O_3 concentration and which may be measured (Gottschalk *et al.*, 2000).

The choice of method of determination is not only dependent on the phase and concentration of the O_3 present but also of the method of generation. Oxides of nitrogen may form when air is used for generation. These oxides may interfere when oxidation, as in several of the above-mentioned methods, is the principle for concentration determinations. In such cases a photometric method may be better suited (Walter & Weber, 1972).

Applications of O_3 in the food and beverage industry

Ozone technology has been utilised as a disinfectant in the oxidation of inorganic and organic compounds (Gottschalk *et al.*, 2000). It now has a prominent function in water treatment and has been employed by certain European countries since the beginning of the twentieth century (Yu & Yu, 2001). Ozone water treatment includes the treatment of drinking and bathing water, and industrial and municipal wastewater.

The development and utilization of O_3 for agricultural and food applications was not recommended by the US Food and Drug Administration (FDA) (Rice, 2001). However, in June 2001, the use of O_3 as an "antimicrobial agent for the treatment, storage and processing of foods in gas and aqueous phases" was approved. The approval was given in response to a "Food Additive Petition" submitted on the 5th of August 2000 by an independent panel of experts in food and O_3 sciences that had been convened by the Electric Power Research Institute. In 1997 this group confirmed that the use of O_3 in direct contact with foods is "Generally Recognised As Safe" (GRAS) when used under "Good Manufacturing Conditions" (Graham, 1997). As a result of this, O_3 has now found wide application in the food and agricultural industries (Rice, 2001).

In agriculture, O_3 has been evaluated for the fumigation of soils and, in the future, will possibly replace methyl bromide (Rice, 2001). This could eventually increase the yield of certain crops. In the food industry O_3 is now being used for the sanitizing of packaging material, and storage and processing facilities (Hampson, 2000). In the meat industry, O_3 is used in the processing facilities. It is furthermore used for ice production and storage and the disinfection of plant workers' shoes (Rice, 2001).

It has been shown that both gaseous O_3 and O_3 dissolved in water can be applied in the stabilisation of the freshness of food. Stabilisation is achieved by the O_3 preventing microbial intervention (Ried & Mielcke, 2001). In the fresh fruit industry, O_3 is used to sterilise process water for fruit and vegetable washing (Xu, 1999). It was also found that the microbiological quality of strawberries could be improved when using O_3 instead of the usual chlorine treatment. In this study, the total microbial and yeast and mould populations were reduced by 72 and 60%, respectively (Steiner & Yuan, 2001).

Since May 1997, when O_3 received GRAS status (Graham, 1997), it has found application in the wine industry (Hampson, 2000). It has been found to be effective in barrel and tank cleaning and sanitation, "cleaning-in-place" systems and general surface sanitation

G. OZONE COMBINED TREATMENTS

Combined treatment strategies are used when a biological or chemical treatment alone is not efficient enough to achieve the required result (Gottschalk *et al.*, 2000). A chemical oxidation process, such as ozonation, may be used to improve biodegradability of compounds in wastewater as a pre-treatment to a biological process.

Fernando *et al.* (2001) reported that ozonation was successfully combined with activated sludge treatment for the treatment of cherry stillage. In this case, ozonation played an important role in the oxidation of the polyphenols that are present in high concentrations in the cherry wastewater.

Martin *et al.* (2002) also investigated ozonation as a pre-treatment combined with anaerobic digestion of vinasse. The function of the ozonation process was to convert refractory and toxic compounds, such as phenol, into simpler molecules with lower molecular masses that could then be used as substrate by the anaerobic populations. In this case, ozonation for 2 h did decrease the COD of the vinasse from 109 200 to 82 100 $mg.L^{-1}$. However, it did not show improved kinetic behavioural results during the anaerobic digestion step. In contrast, it was found that when vinasse was treated with O_3 in the presence of UV-light and titanium oxide, the specific rate of CH_4 production ($mL\ CH_4/g^{-1}VSS.h^{-1}$) did increase by a factor of 1.25 (from 3.56 to 4.50) compared to the untreated wastewater.

Benitez *et al.* (1999) investigated the purification kinetics of winery wastes in separate ozonation and anaerobic digestion studies and in a combined ozonation pre-treatment anaerobic digestion process in a magnetically stirred batch digestion unit. It was found that the winery waste COD could be reduced by 20% using just ozonation. When ozonation was combined with oxidants, UV radiation and hydrogen peroxide, the COD reduction increased to 30%. A CH_4

yield coefficient of 187 mL $CH_4.g^{-1}$ COD degraded and COD removal efficiencies of more than 98%, could be attained in the separate anaerobic treatment set-up. The combined ozonation pre-treatment and anaerobic digestion further increased the CH_4 yield coefficient to 215 mL $CH_4.g^{-1}$ COD degraded.

A study in Greece showed that anaerobic digestion in an UASB reactor can decrease the organic load of "currant"-wastewater by 95.5% yielding an effluent with a COD of only 185 $mg.L^{-1}$ (Athanasopoulos & Athanasopoulos, 1998). This value was still well above the Greek legal limit of 50 $mg.L^{-1}$ for underground discharge. A post treatment with O_3 for 3 h at a concentration of 300 $mg.h^{-1}$ reduced the COD to the legal limit.

Sigge *et al.* (2001) also investigated the feasibility of using a combination of O_3 as a post-treatment and UASB as primary treatment with fruit cannery wastewater as substrate feed. The anaerobic treatment reduced the COD by 90 to 93% yielding an effluent with a COD of 315 to 450 $mg.L^{-1}$. This final COD value was then reduced by 25% after a 5 min, and 53% after 60 min ozonation respectively, lowering the COD to approximately 236 to 338 $mg.L^{-1}$ for the former and 149 to 212 $mg.L^{-1}$ for the latter ozonation times.

In another study by Sigge *et al.* (2002), anaerobic treatment in an UASB reactor was found to again lower the COD of cannery and cellar wastewater by 90 to 93% and 90 to 96%, respectively. A post-ozonation combination using a granular activated carbon column led to further COD reductions: the COD of the cannery wastewater was reduced by 27% after 5 min and 53% after 30 min ozonation. This led to final COD values of 331 to 473 $mg.L^{-1}$ and 247 to 353 $mg.L^{-1}$ after 5 and 30 min ozonation, respectively. In the case of the cellar wastewater, the COD was reduced by 30% after 5 min and 55% after 30 min ozonation. Here the final COD values were 104 to 259 $mg.L^{-1}$ and 67 to 167 $mg.L^{-1}$ after 5 and 30 min ozonation, respectively.

H. SUMMARY

The classical applications of AD applied since the end of the 19th century included the treatment of household wastewater in septic tanks, slurries in digesters and sewage sludge in municipal treatment plants (Van Lier *et al.*, 2001). Reactors

were limited to the continuous stirred types requiring lengthy treatment retention times. It was not until the 1960s that design concepts were developed, which could allow for biomass accumulation at relatively low HRTs (Iza, 1991). In 1997, the data bank of anaerobic reactors included 1066 full-scale operating anaerobic reactors worldwide: 956 (89.7%) used for industrial wastewater, 78 (7.3%) used for domestic sewage and 32 (3.0%) for organic solid waste treatment (Anon., 1997).

Compared to aerobic treatment processes, the operation and maintenance of anaerobic treatment processes are relatively low (Hulshoff Pol & Lettinga, 1986). AD does not have the excessive costs of aeration and although anaerobic breakdown is considerable slower than aerobic breakdown, it converts a larger fraction of organic carbon to gases. The CH_4 in the produced biogas can be used as energy source for heating and pumping in the wastewater treatment system. Another important factor is that in AD only 10% of the carbon is formed as sludge in comparison to the 90% produced by aerobic treatment (Nazaroff & Alvarez-Cohen, 2001). One can expect greater financial benefits of the choice of AD as wastewater treatment option to grow strongly as a result of the constant escalation of charges levied by regional water authorities, and increasing public concern in terms of water pollution and its impact on the environment.

Of the various anaerobic treatment systems, the UASB process has found the widest application (Lettinga & Hulshoff Pol, 1991; Van Lier *et al.*, 2001). The UASB process displays excellent sludge retention by the phenomenon of granulation (Schmidt & Ahring, 1996). Very little is, however, known about the mechanism of granule formation and maintenance or disintegration of granules (Kosaric *et al.*, 1990). Various studies have been conducted to find methods to improve the granulation process (Verstraete & Vandevivere, 1999). The additions of cationic polymers, divalent cations and granulation nuclei have all been shown to be successful (Anon., 1997). It has been shown that the type of wastewater treated influences the microstructure of the granule, including the microbial diversity of the acidogenic populations (Britz *et al.*, 1994) and the microbial structure and activity (Fang *et al.*, 1994). The effect that toxicants present in wastewater, such as phenol, cyanide and sulphur, have on biogranules and UASB performance has also been investigated (Fang & Chan, 1997; Gijzen, H.J. 2000; Lin *et al.*, 2001).

The treatment of industrial wastewater using a UASB reactor is considered a proven technology (Lettinga & Hulshoff Pol, 1991). Optimisation of reactor configuration might however, still be further investigated (Van Lier *et al.*, 2001).

Although AD is efficient, as with most other biological processes, treatment is often not sufficient enough to reach legislative standards (Gottschalk *et al.*, 2000). This will thus call for either or both pre- and post-treatment.

The use of O_3 in food is a relatively new technology as it was only FDA approved in June 2001. In contrast, O_3 has been used in water treatment, mostly as a sanitizer, for more than 90 years (Rice, 2001). The improvement in O_3 generators and better controls as well as the fact that ozonation does not leave any residual compounds (Graham, 1997) makes ozonation an excellent option for pre- and post-treatments to anaerobic digestion. Ozonation can be employed successfully as a pre-treatment to increase biodegradability and convert refractory and toxic compounds into simpler molecules (Gottschalk *et al.*, 2000; Martin *et al.*, 2002). Ozonation as post-treatment has also been shown to be a feasible method to polish effluents so as to reach legislative discharge standards (Athanasopoulos & Athanasopoulos, 1998; Sigge *et al.*, 2001; Sigge *et al.*, 2002).

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CHAPTER 3

OPERATIONAL OPTIMISATION OF AN UASB BIOREACTOR TREATING CELLAR WASTEWATER

Summary

A mesophylic, laboratory-scale upflow anaerobic sludge bed (UASB) bioreactor was evaluated for the treatment of cellar wastewaters. The bioreactor was subjected to an acclimatisation period at a hydraulic retention time (HRT) of 24 h and the substrate pH adjusted to 8.0 to avoid rapid acidification. Extreme variations in effluent pH and chemical oxygen demand (COD) removal efficiencies between 29 and 64% were experienced. An addition of strains of *Acinetobacter haemolyticus*, *Burkholderia cepacia* and *Cryseomonas luteola* isolated from raw cellar effluent to the substrate did not lead to greatly improved performance. Chemical oxygen demand removal efficiencies with variations of 35 to 77% were experienced. An increase in organic loading rate (OLR) by increasing the substrate COD led to the COD removal stabilising above 60%. The substrate pH was lowered from 8.00 to 5.73 while the HRT was kept at 24 h to find the lowest efficient operational pH. At substrate pH 7.5 stable-state in terms of COD removal and effluent pH was reached. The lowest efficient operational pH was found to be 5.73 (substrate COD < 5 000 $mg.L^{-1}$). In a further study at pH 6.0 the HRT was reduced from 24 to 17.4 h and subsequently the OLR increased from 8.39 to 10.95 kg $COD.m^{-3}d^{-1}$. The most efficient operational HRT was found to be at 19.7 h (COD removal efficiency = 84%). Lower HRTs led to severe reactor acidification and granule wash-out. The economic implication of the UASB functioning at a low pH, in terms of neutralisation costs, and a relatively short HRT are considerable.

Introduction

The wine industry significantly contributes to South Africa's pollution problems. This also includes the increased salination and eutrofication of water resources and ultimately soil degradation (Van Schoor, 2000). Water usage for wine-making ranges from 700 to 3 800 L per ton of grapes processed and 70% of the water intake is returned as wastewater. Typical, nutrient deficient, acidic cellar wastewaters show considerable compositional variation with average chemical oxygen demand (COD) values varying from 800 to 12 800 $mg.L^{-1}$ (Petruccioli *et al.*, 2002). This variation in COD values together with high penalties for not complying to set discharge standards and the necessity to remain competitive in an increasingly globalised market place (Hayward *et al.*, 2000) has forced cellars to consider various wastewater treatment options.

Anaerobic digestion (AD) as a treatment option offers several advantages: it can be used on small to very large scale (Lin & Yang, 1991; Lettinga, 2001); has relative low operational and maintenance costs; produces far less biomass than aerobic systems; and produces biogas which can be utilised as an energy source for the heating of the anaerobic reactor (Nazaroff & Alvarez-Cohen, 2001). A specific application of this process, namely the upflow anaerobic sludge blanket (UASB) system, developed by Lettinga and co-workers in the 1970's (Lettinga & Hulshoff Pol, 1991), offers the added advantage of biomass being retained within the system. This may lead to high removal efficiencies at high volumetric loading rates and relatively short hydraulic retention times (HRT) (Hickey *et al.*, 1991; Schimdt & Ahring, 1996; Van Lier *et al.*, 2001).

The use of the UASB bioreactor design has in the past been shown to be successful in the treatment of various food industry wastewaters including those from maize, meat and dairy processing, brewery activities and fruit cannery wastewaters (Ross, 1989; Strydom *et al.*, 1997; Puñal & Lema, 1999; Sigge *et al.*, 2002). Since wastewaters from the alcoholic fermentation industry, such as cellar and distillery wastewaters, are also highly polluted, the anaerobic digestion technology may be applied as a treatment option (Driessen *et al.*, 1994; O'Kennedy, 2000). UASB treatment has already been shown to be feasible for the treatment of cellar wastewaters (Andreottola *et al.*, 1998; Müller, 1998; Ronquest & Britz, 1999; Sigge *et al.*, 2002).

Knowledge of the natural microbial community in a wastewater and subsequent enhancement of this community could possibly be utilised to improve the start-up period and overall efficiency of the anaerobic digestion process (Van der Merwe & Britz, 1994). Britz *et al.* (2002) found that conditions favouring the growth of propionic-acid-bacteria, like *Propionibacterium*, could enhance the granulation process. It has also been shown that the inoculation of the substrate with certain selected bacteria (Keyser *et al.*, 2003) or moulds (Jiménez *et al.*, 2003) enhanced the degradation process. Keyser *et al.* (2003) also reported that the incorporation of a strain of *Enterobacter sakazakii* into anaerobic granules led to an improved treatment specificity of cellar wastewater.

The aim of this research was to evaluate the use of a laboratory scale mesophylic UASB system as a treatment option of cellar wastewater. This will involve a comprehensive stabilisation study including nutrient additions, substrate pre-degradation by microbial enhancement, and the heat treatment of the raw substrate. In a second phase, the reactor will be optimised in terms of lowering the substrate pH so as to minimise neutralisation costs. A third phase will involve the determination of the highest organic loading rate (OLR) at which the best overall operational efficiency could be maintained.

Materials and methods

Bioreactor

A laboratory scale UASB bioreactor with a height of 1 m, diameter of 50 mm and an operational volume of 2.3 L, was used (Fig. 1). The substrate was pulse-fed from the bottom of the reactor using a peristaltic pump (Watson-Marlow 101U/R) controlled by an electronic timer and the biogas exited at the top via an open gas/solid separator. The volume of biogas produced was determined using a manometric unit fitted with a gas-tight valve and an electronic counter. The overflow of the bioreactor drained through a U-shaped tube to prevent atmospheric oxygen entering into the system. The upflow velocity was set at 2.8 $m.h^{-1}$, using a Watson Marlow 302S pump for re-circulation, which also kept the granular sludge in suspension. Insulation, heating tape and an electronic

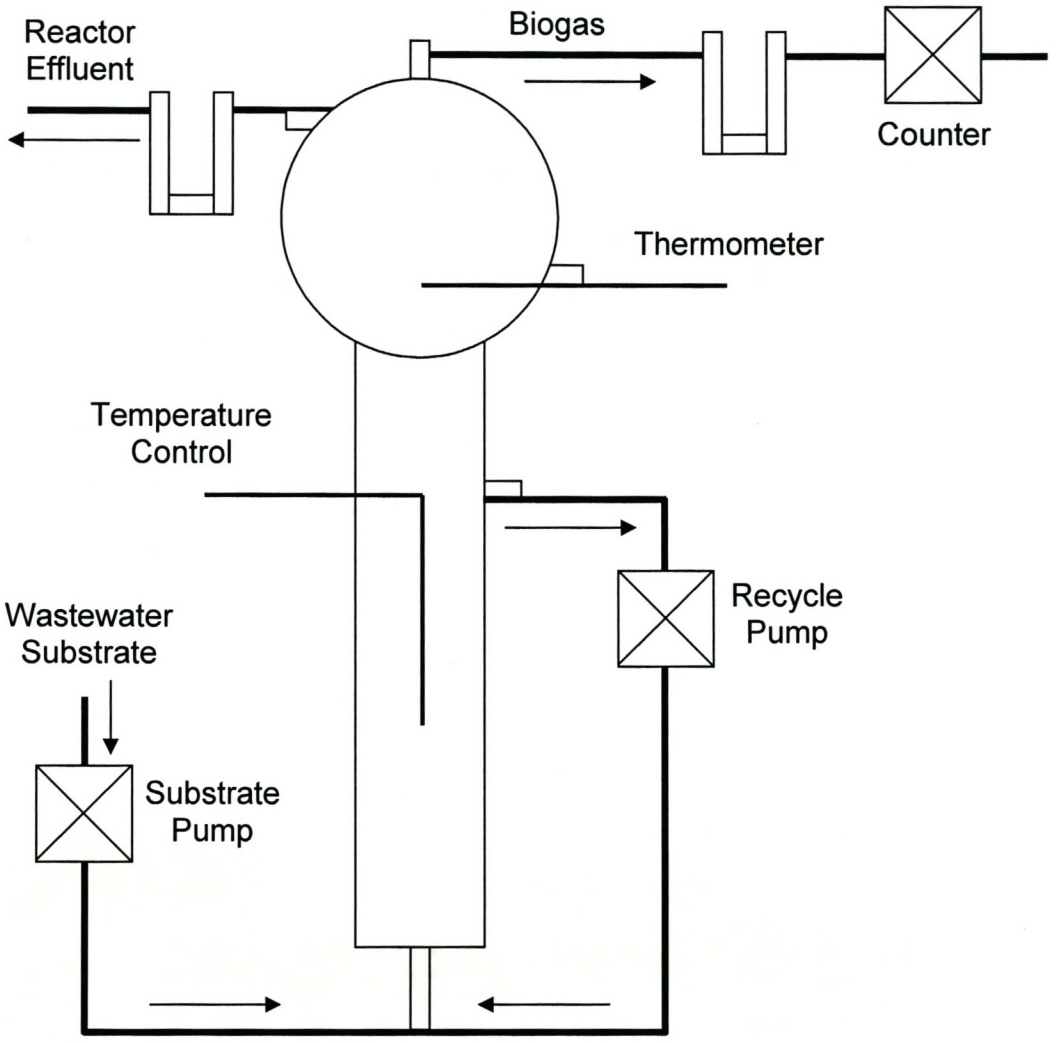

Figure 1. Laboratory-scale upflow anaerobic sludge blanket bioreactor.

control unit were used to maintain the operational temperature at 35°C (Meyer *et al.*, 1983).

Bioreactor start-up

The bioreactor was seeded with a mixed anaerobic granule consortium, obtained from the Department of Food Science, University of Stellenbosch to a settled height of 300 mm (30% of the reactor height). The reactor was allowed to stabilise for 24 h while feeding water supplemented with 500 $mg.L^{-1}$ each of urea and K_2HPO_4. The pH was adjusted to 8.0 using 1M KOH. The hydraulic retention time (HRT) was set at 24 h and a granule acclimatisation period was implemented with a diluted cellar wastewater as substrate (COD of 2 000 $mg.L^{-1}$).

Substrate

The cellar wastewater was obtained from the Rupert and Rothschild Fredericksburg cellar (R-RF) (Simondium, South Africa) during the period April 2002 to March 2003. The substrate batches, which included wastewater from the vintage and non-vintage seasons, were kept at -18°C in 25 L containers. When required, individual containers were defrosted and then stored at 4°C. The cellar wastewater was diluted to the desired COD level and as cellar wastewater is a nutrient deficient substrate (Water Research Commission, 1993) the diluted wastewater was supplemented in excess with the following nutrients when used as bioreactor substrate: 500 $mg.L^{-1}$ urea and 200 $mg.L^{-1}$ K_2HPO_4 to prevent N and P limitations; and 500 $mg.L^{-1}$ sucrose, from day 83 onwards, to stimulate reactor performance. From day 30 onwards it was also supplemented with 500 $mg.L^{-1}$ $KHCO_3$ to increase alkalinity. Substrate pH was adjusted as required for each study (8.00 to 5.73) using 1 M KOH.

Analytical methods

The following operational parameters were monitored according to Standard Methods (APHA, 1992): pH; alkalinity; total suspended solids (TSS); and total volatile suspended solids (VSS). Chemical oxygen demand (COD), orthophosphate phosphorous (PO_4), and total Kjeldahl nitrogen (TKN) were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1992).

Dissolved organic carbon (DOC) was determined by Environmentek, CSIR, Stellenbosch, using an automated Persulphate-Ultraviolet oxidation method based on the colorimetric detection of CO_2 generated (Mike Louw, 2002, personal communication).

Polyphenols were determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). The volatile fatty acids (VFA) were determined on a Varian (Model 3700) gas chromatograph, equipped with a flame ionisation detector and a 30 m fused silica capillary column with FFAP bonded stationary phase (Quadrex Co. New Haven). The column temperature was first held at 105°C for 2 min, and then increased at a rate of 8°C per min to 190°C. The detector and inlet temperatures were set at 300° and 130°C, respectively, and nitrogen gas was used as carrier gas at a flow rate of 6.1 $mL.min^{-1}$.

A Fisions 3 300 gas chromatograph equipped with a thermal conductivity detector and 2.0 m x 3.0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh, was used to determine biogas composition. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 30 $mL.min^{-1}$.

Microbial isolations

Cellar effluent media (CE-medium), consisting of 20% (v/v) cellar effluent, 80% water (v/v), 200 $mg.L^{-1}$ each of urea and K_2HPO_4, was prepared and 14 $g.L^{-1}$ agar (Merck) was added. Pour plate dilution series were carried out on the R-RF cellar wastewater "batch 2" and a cellar wastewater obtained from the Bergkelder, Distell cellar (Stellenbosch, South Africa). The plates were incubated aerobically and anaerobically at 35°C for 48 h.

Prevalent strains from the 10^{-5} dilution range for both wastewaters were inoculated into 10 mL sterile CE-medium and then incubated at 35°C for 48 h. One mL of this medium was then used to inoculate 9 mL sterile CE-medium, incubated at 35°C for 48 h so as to monitor the growth of the dominant species. Every second day, 1 mL of the latest inoculated medium was used to inoculate a further 9 mL sterile CE-medium, which was again incubated at 35°C for 48 h. After 14 days, the best strains were streaked out onto CE-agar until pure colonies were obtained. The morphology of each of the isolates was determined by bright field microscopy of Gram preparations. The purified isolates were characterised

using catalase and oxidase character tests and combinations of the API 20E and 20NE systems (bioMérieux sa, 69280 Marcy l'Etoile, France).

Experimental study I: General bioreactor stabilisation

In the first study the reactor performance was stabilised while feeding a substrate with the pH adjusted to 8.0. An increase in alkalinity facilitated by $KHCO_3$ additions from day 30 (500 $mg.L^{-1}$), sucrose supplementations (500 $mg.L^{-1}$) to the substrate from day 83, heat-treated substrate from days 113 to 192 and microbial supplementation from days 120 to 170, were investigated as methods to enhance bioreactor stability.

The heat treatment, used to improve biodegradability, involved the autoclaving of the substrate (100 kPa at 121°C for 15 min). The microbial supplementation process used to enhance digestion of cellar wastewater prior to anaerobic digestion involved additions of mixtures of the isolated bacteria (>10^8 $cfu.mL^{-1}$), grown for 24 h at 35°C in 10 mL CE-media, to the 2.3 L reactor substrate.

Experimental study II: Lowering of substrate pH

In this study the substrate pH was reduced from 8.00 to 5.73 in 7 steps. The HRT and substrate CODs were kept constant at 24 h and 3 440 $mg.L^{-1}$ (min = 2 219 and max = 4 855 $mg.L^{-1}$), respectively.

Experimental study III: Increase in OLR

The third study was divided into two phases. In the first phase the OLR was increased by increasing the substrate COD from *ca.* 3 000 to 8 000 $mg.L^{-1}$. Substrate pH was kept at 5.73 from days 357 to 396 and adjusted to 6.00 on day 397 to prevent possible system failure. In the second phase, the OLR was further increased by the shortening of the HRT from 24 to 17.6 h in five steps. The substrate pH was kept constant at 6.00 throughout the second phase of this study.

Results and discussion

Wastewater Composition

The compositions of the cellar wastewater batches used in this study are shown in Table 1. It is evident from the data that the wastewater showed considerable variation in organic content (measured as COD) and the pH with variations from 3 492 to 10 206 $mg.L^{-1}$ and 3.6 to 6.3, respectively. This was, however, expected as cellar wastewater varies according to cellar operation during especially the harvest and post-harvest periods (Van Schoor, 2000). The data also showed low nitrogen and phosphorous content necessitating wastewater N and P supplementation before use as reactor substrate.

Study I: General bioreactor stabilisation

The performance of the UASB bioreactor over the first 278 days is shown in Fig. 2. As the cellar wastewater batches displayed large variations in total and soluble COD and the solid content (Table 1), it was difficult to standardise the wastewater prior to substrate preparation. Furthermore, the wastewater batches were not stored under sterile conditions and breakdown occurred throughout the storing period. This contributed to the difficulties experienced during the standardising of the substrate COD.

During the first 8 d of the study, a diluted cellar wastewater substrate with a pH adjusted to 8.0 and COD of 2 000 $mg.L^{-1}$, was fed to the UASB reactor. This led to a drop in effluent pH from 7.60 to 5.25. Therefore, on day 9 the inlet feed was further diluted to obtain a substrate with a COD of *ca.* 1 000 $mg.L^{-1}$. Reactor performance did not improve (COD removal efficiency = 52 to 69%), and subsequently the HRT was lengthened from 24 to 41.7 h on day 21. In order to increase the reactor alkalinity to try and stabilise the reactor buffering capacity, $KHCO_3$ additions were made daily from day 30 onwards. The COD removal efficiency during this period (days 30 to 82) varied considerably (28 to 64%).

Reactor instability displayed by the large variation in COD removal efficiency during this phase led to other stimulation methods being considered. Since cellar wastewater is carbohydrate deficient as it results from a yeast fermentation process, sucrose additions of 500 $mg.L^{-1}$ were made to the wastewater from day 83 onwards. The COD removal efficiency improved from

Table 1. Composition of the raw wastewater batches, obtained from the Rupert and Rothschild Fredericksburg cellar used as substrate for the UASB bioreactor

Parameter		R-RF Batch			
		1	**2**	**3**	**4**
COD_{total} ($mg.L^{-1}$)		5 094	3 533	3 492	10 206
		±SD 309	±SD 865	±SD 432	±SD 794
$COD_{soluble}$ ($mg.L^{-1}$)		3 852	2 690	2 376	9 936
DOC ($mg.L^{-1}$)		1 010	n.d.	651	2 481
pH		6.2	6.3	3.6	3.6
Phosphorous (mg $PO_4.L^{-1}$)		26	20	14	39
TSS ($mg.L^{-1}$)		426	648	639	572
VSS ($mg.L^{-1}$)		326	562	360	507
TKN ($mg.L^{-1}$)		6.25	n.d.	0.00	5.83
Conductivity ($\mu S.s^{-1}$)		818	n.d.	1 040	1 390
Polyphenols (mg gallic acid equivalents.L^{-1})		32	n.d.	15	50
VFA ($mg.L^{-1}$):	Acetic	62	n.d.	15	109
	Propionic	18	n.d.	17	59
	Butyric	0	n.d.	10	0
	Valeric	0	n.d.	6	0
	Total VFAs	80	n.d.	48	168

n.d. = not determined

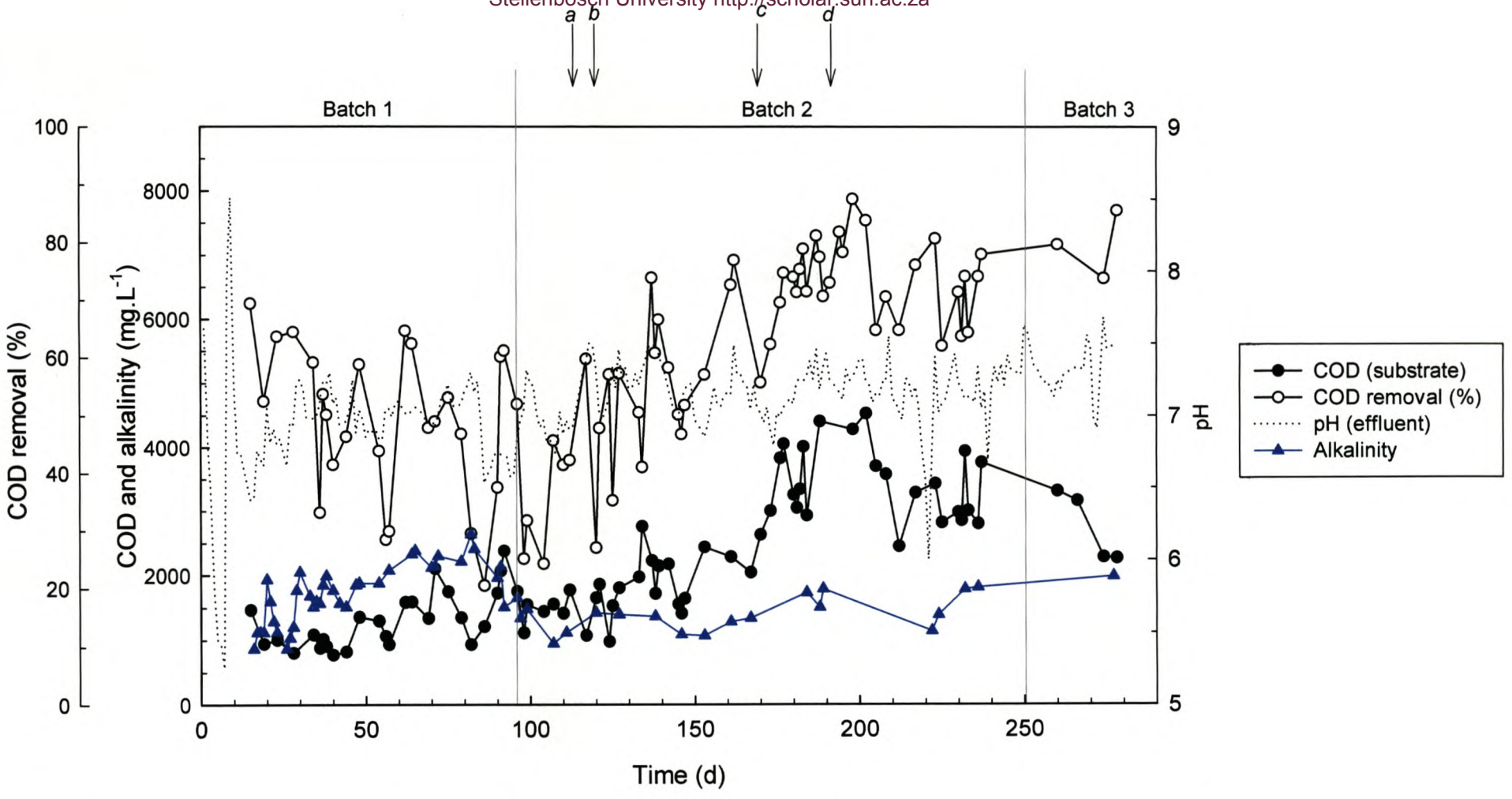

Figure 2. Reactor performance during the stabilisation study: a) start of substrate heat treatment; b) start of pre-degradation; c) end of pre-degradation; and d) end of substrate heat treatment.

20% on day 86 to 61% on day 92. However, COD removal efficiency again decreased and the data showed the continuing varying of COD removal efficiency pattern similar to that observed prior to sucrose additions. In a second attempt to stimulate the COD removal efficiency, the OLR was increased by the HRT being lowered to 24 h on day 113. The COD removal continued to vary as before.

In order to try to improve the reactor biodegradability, the substrate was then subjected to a heat-treatment process from days 113 to 192. As can be seen (Fig. 2), COD removal efficiency continued to be inconsistent, but in total, the COD removal efficiency by day 191 had increased to 73%. It is not certain whether this increase can be attributed directly to the heat treatment process.

During the same period (days 120 to 170), pre-degradation and possible bioaugmentation of the reactor with bacteria isolated from cellar effluents were attempted. Additionally, the substrate COD was also gradually increased from 2 200 $mg.L^{-1}$ on day 142 to 4 400 $mg.L^{-1}$ by day 188. Once the heat-treatment was terminated, a slight decrease in COD removal efficiency was observed. The termination was, however, coupled to a decrease in substrate COD, which also could have led to the slight decrease in COD removal efficiency.

In the past it has been shown that the inoculation of the reactor substrate with bacteria naturally occurring and actively degrading the organic compounds in the original wastewater, could improve the anaerobic digestion process (Keyser *et al.*, 2003). Based on the above, three different dominant bacterial strains were isolated from the cellar wastewater, characterised and identified (Table 2) as *Acinetobacer haemolyticus*, *Burkholderia cepacia* and *Cryseomonas luteola*. The level of the API Systems identification is given in Table 3. All three isolates grew well on CE-agar. When streaked out on this agar, colonies could be obtained within 24 h of incubation at 35°C.

Cellar effluent medium containing mixtures of the three isolated bacteria (>10^8 $cfu.mL^{-1}$) were added to the reactor substrate from day 120. Since COD removal efficiency remained variable during the complete microbial supplementation study (35 – 77%), the additions were terminated on day 170.

The increase in substrate COD from 2 635 $mg.L^{-1}$ on day 170 to 4 055 $mg.L^{-1}$ by day 177 (Fig. 2) as a result of a decrease in the dilution factor most probably led to the increase in COD removal efficiency. The average COD

Table 2. Characteristics of cellar wastewater isolates using the API 20E and 20NE identification systems

Test		Strains		
		Type 1	Type 2	Type 3
Gram		-	-	-
Catalase		+	+	+
Morphology		cocci (single)	rods (single)	rods with rounded edges (single)
Oxidase		-	-	-
API 20 E				
ONPG		-	+	+
Arginine dihydrolase		-	+	+
Lysine decarboxylase		-	+	-
Orthinine decarboxylase		-	-	-
Citrate utilization		-	+	+
H_2S production		-	-	-
Urease		-	-	-
Tryptophane desaminase		-	-	-
Indole production		-	-	-
Acetoin production		+/-	+	-
Gelatinase		-	-	-
Fermentation of:	glucose	-	+	+
	mannitol	-	+	+
	inositol	-	+	+/-
	sorbitol	-	+	+
	rhamnose	-	+	+
	sucrose	-	+	+
	melibiose	-	+	+
	amygdalin	-	+	+/-
	arabinose	-	+	+
API 20 NE				
NO_3		-	+	+
Indole production		-	-	-
Glucose acidification		+	+	+
Arginine dihydrolase		-	-	-
Urease		-	-	-
Esculin hydrolysis		+/-	+/-	+
Gelatine hydrolysis		-	-	-
PNPG		-	-	+
Assimilation of:	glucose	-	+	+
	arabinose	-	+	+
	mannose	-	+	+
	mannitol	-	+	+/-
	N-acetyl-glucosamine	-	+	+
	maltose	-	+	+
	gluconate	-	+	+
	caprate	+	+	-
	adipate	-	+	-
	malate	+	+	+
	citrate	+	+	+
	phenyl-acetate	-	+	-

Table 3. Identification using the API NE System of the prevalent strains present in cellar wastewater

Strain Type	Identification	Percentage ID
1	*Acinetobacter haemolyticus*	89.7
2	*Burkholderia cepacia*	99.9
3	*Cryseomonas luteola*	97.9

removal prior to the increase was 55% (days 120 to 170) compared to 77% (days 180 to 202) after the increase. The possibility of the higher substrate load stimulating COD removal efficiency should be considered as it was found that lower substrate CODs generally led to lower COD removal efficiencies (days 170 to 208).

From day 170 the bioreactor was regarded as stable except for a drop in pH on day 219, which was attributed to temperature control problems. The COD removal efficiency and alkalinity however, remained above 70% and 1 000 mg $CaCO_3.L^{-1}$, respectively. Once the temperature was adjusted to 35°C again, the bioreactor recovered to its performance prior to the temperature control problem. The reactor continued with this performance after the introduction of substrate batch 3 on day 251. It was found that the effluent pH varied between 7.2 and 7.6 and COD removal was above 74%.

Stable-state is defined as a state, which can be maintained indefinitely without system failure (Cobb & Hill, 1990), during which the variation in bioreactor performance parameters is less than 10%. Although this state, by definition, was not always reached, the reactor displayed a relatively good performance and it was thus decided to proceed with Study II.

Study II: Lowering of substrate pH

Wastewaters discharged from cellars often have a pH as low as 3.6 (Table 1). To prevent stressing the microbial population of the bioreactor, especially the methanogenic population that functions optimally at pH 6.7 to 7.4 (Bitton, 1999), the pH should be neutralised before introducing the wastewater to the reactor. However, neutralisation contributes greatly to the operational costs of the UASB treatment process. For economic reasons it was decided to find the lowest operational pH and subsequently acclimatise the microbes present in the bioreactor to lower substrate pHs.

Substrate pH reduction was implemented on the UASB reactor after it showed relatively good performance in terms of COD removal efficiency and effluent pH stability with the substrate pH adjusted to 8.0 (Fig. 2). The COD removal efficiencies remained higher than 70% for days 177 to 278 before lowering of the reactor pH commenced. During the same period (days 177 to 278) the average alkalinity was 1 650 mg CO_3L^{-1} (min. = 1 100 mg CO_3L^{-1}). An

alkalinity of above 1 000 mg CO_3L^{-1} is regarded as sufficient for the digestion to proceed satisfactorily and serve as a buffer to prevent a sudden decrease in reactor pH (Tchobanoglous & Burton, 1991).

The substrate pH was lowered from 8.0 to 7.5 on day 279 (Fig. 3). Substrate pH was kept at 7.5 for 9 days and, as can be seen from Fig. 3, the COD removal increased from ca. 74% to an average of ca. 88% during this period. On day 288 the substrate pH was lowered to 7.0 and COD removal decreased to 80%, but rapidly recovered to 89% by day 292. Since the reactor effluent pH remained above 7.1, the substrate pH was further decreased to 6.5 on day 294.

The effluent pH was found to increase and remain above 7.2, and COD removal efficiency increased to 91% by day 299. After a further 7 d the substrate pH was reduced to 6.0. The effluent still showed a relatively high average pH of 7.3 and the COD removal efficiency was stable between 86 and 90%. The effectiveness of the bioreactor could further be illustrated by the low VFAs of 159 and 124 $mg.L^{-1}$ in the effluent on days 307 and 310, respectively. Reduction of the substrate pH to 5.8 on day 315 did lead to a decrease in average effluent pH to 7.1. This was, however, well above the pH of 6.5 where methanogenesis is slowed and eventually terminated (Nazaroff & Alvarez-Cohen, 2001). At this time the VFA-concentration increased to 205 $mg.L^{-1}$ by day 318 and 524 $mg.L^{-1}$ by day 321. By day 323, the bioreactor was regarded as stabilised, as effluent pH remained constant and COD removal efficiency increased to 90%. The VFA-concentration also decreased to 105 $mg.L^{-1}$ by day 324.

The setting of the substrate pH at 5.75 on day 324 did not initially affect the effluent pH. The excellent reactor performance at this stage was highlighted by VFAs of 55 $mg.L^{-1}$ on day 328 and 106 $mg.L^{-1}$ on day 331. At the same time a COD removal efficiency of 91% was achieved. It was found that the reactor performance increased at a substrate pH close to neutral pH. This could probably be ascribed to the methanogenic populations functioning optimally at a pH of 7.0 to 7.2 (Bitton, 1999).

On day 329 a new batch of wastewater, batch 4, was introduced to the reactor at an average COD concentration of 3 800 $mg.L^{-1}$. The effluent pH decreased to an average of 7.03 and the VFAs on day 335 were measured as 234 $mg.L^{-1}$. The COD removal efficiency remained above 85% and a further

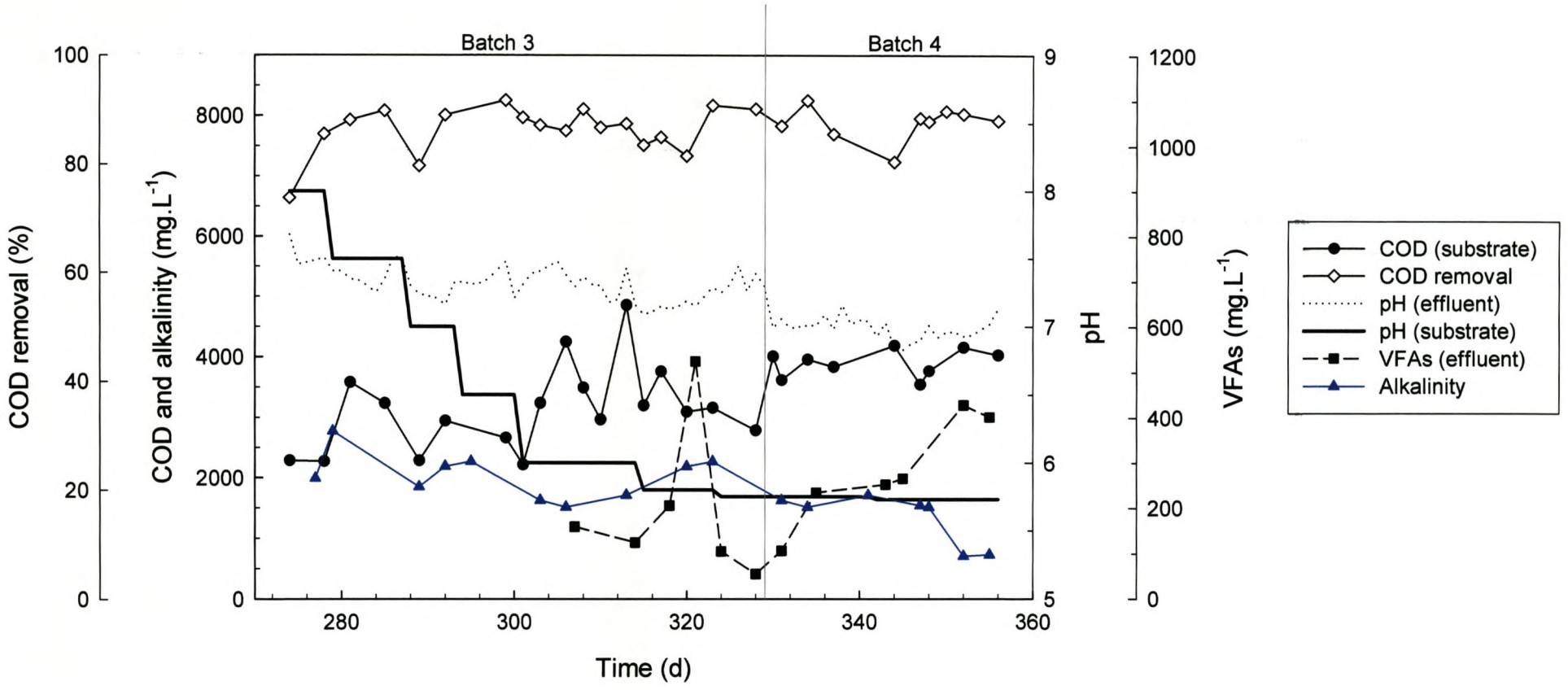

Figure 3. Reactor performance during the substrate pH reduction study from days 274 to 356.

reduction of the substrate pH to 5.73 on day 342 led to the COD removal efficiency being reduced to 80% on day 344. The reactor once again stabilised in terms of COD removal efficiency within 3 days and the average COD removal from day 347 until the end of the second study on day 356, was 88%. However, the low substrate pH still led to a high VFA-concentration of 415 $mg.L^{-1}$ on day 352. Since the effluent pH now remained below 7.0 and the VFA-concentration was above 400 $mg.L^{-1}$, it was decided to terminate this study and regard 5.73 as the lowest efficient operational substrate pH. The economic implications regarding savings in neutralisation costs as a result of the use of a lower substrate pH should be significant.

Study III - Increase in organic loading rate

(a) Increasing substrate COD: During the original batch collection period (Table 1) it was found that when cellar wastewater is discharged, it can have a COD of as high as 10 000 $mg.L^{-1}$, which when treating this type of wastewater could lead to drastic overloading and reactor failure. It was decided to increase the substrate organic load by decreasing the dilution factor of the reactor substrate (days 357 to 450) (Fig. 4) and to optimise the reactor in terms of OLR. This study was started directly after the completion of Study II and the substrate pH and HRT kept at 5.73 and 24 h, respectively.

From days 357 to 364 (Fig. 4), the substrate COD was increased stepwise from 3 359 to 5 087 $mg.L^{-1}$. During this period the alkalinity remained constant at 1 631 mg $CaCO_3.L^{-1}$ and the average VFA-concentration was 228 $mg.L^{-1}$ (Fig. 5). Chemical oxygen demand removal efficiencies of more than 87% were maintained. When the substrate COD was increased to 5 551 $mg.L^{-1}$ on day 370, the COD removal efficiency and effluent pH dropped to 80% and 6.85, respectively by day 373. Although COD removal efficiency improved to 86% on day 377, the effluent pH was still relatively low at 6.63. By keeping the substrate COD above 5 000 $mg.L^{-1}$, a decrease in the average COD removal efficiency to 82% (days 383 to 394) and VFA-concentration to 536 $mg.L^{-1}$ by day 387, respectively (Fig. 5), was found. A drop in alkalinity over the same period, indicated reactor instability (Fig. 5). The large increase in substrate load probably stressed the methanogenic population inside the reactor (Bitton, 1999). During this time high concentrations

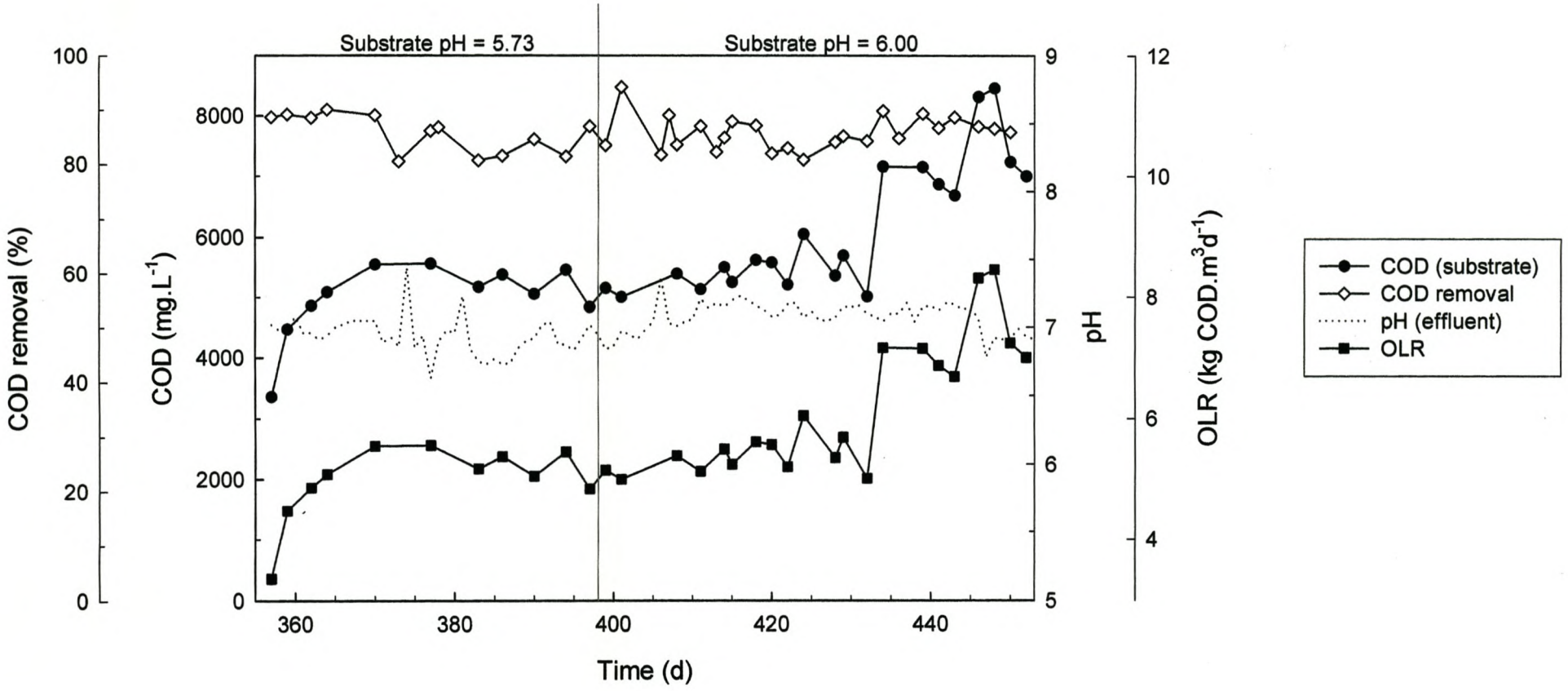

Figure 4. Reactor performance during the increase in substrate COD between days 357 and 452.

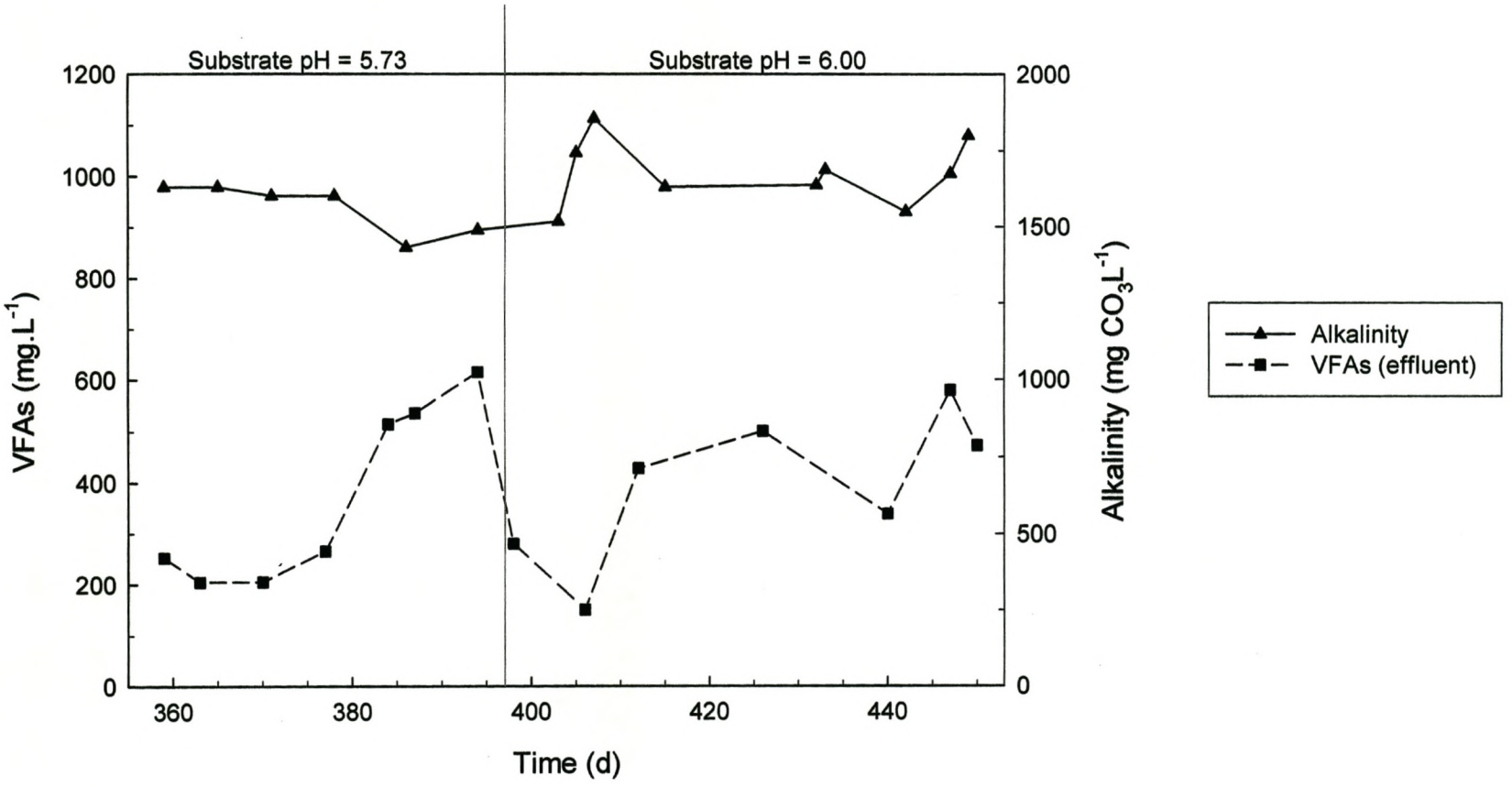

Figure 5. Reactor performance in terms of VFAs and alkalinity during the increase in substrate COD between days 357 and 452.

of VFAs were produced but not effectively removed. Since methanogenic activity was inhibited, bicarbonates were not produced and this led to the decrease in alkalinity.

On day 394, 30 days after the initial COD substrate load increase, the bioreactor had not regained previous removal efficiencies and the VFA-concentration increased to 615 $mg.L^{-1}$. As further increases in substrate COD at an input substrate pH of 5.73 would probably have led to system failure, the substrate pH was reset to 6.0 on day 397 and retained at this pH for the remainder of the study.

The increased substrate pH led to an almost immediate recovery of reactor performance as the VFA-concentration decreased to 281 $mg.L^{-1}$ on day 398. By day 432, the substrate COD varied between 4 838 and 6 048 $mg.L^{-1}$ and an average COD removal efficiency of 85% was maintained. On two separate occasions, days 412 and 426, the VFA-concentration was measured and found to be 428 and 501 $mg.L^{-1}$, respectively (Fig. 5). This did not appear to impact the reactor performance and negatively as the effluent pH always remained above 7.0 with an average alkalinity of 1 667 mg $CaCO_3.L^{-1}$. On day 434 the substrate COD was increased to 7 160 $mg.L^{-1}$. The effluent pH remained above 7.0 and the average COD removal for this period was 88% (days 434 – 443). When the substrate COD was however, increased to 8 316 $mg.L^{-1}$ on day 446, the pH of the effluent dropped to 6.78 (Fig. 4) and VFA-concentration increased to 580 $mg.L^{-1}$ (Fig. 5). Again the relatively high substrate load led to increased stress on the methanogens and accordingly the build-up of volatile fatty acids. The pH however, recovered to 6.92 by day 447, VFAs decreased to 473 $mg.L^{-1}$ by day 450 and COD removal stabilised at an average of 86%. The pH of the effluent was now considerably lower than when substrates with lower CODs (<8 000 $mg.L^{-1}$) were fed. For the remainder of the study it was decided to only increase the OLR by reducing the HRT.

(b) Reducing the HRT: For economic reasons, it is important to treat wastewater as quickly and effectively as possible. It was necessary to find the shortest possible HRT where efficient degradation by the reactor microbial population was still possible. In this study the HRT was shortened from 24 to 17.4 h in 5 consecutive steps (Table 4) with a subsequent increase in OLR from 8.39 to 10.95 kg $COD.m^{-3}d^{-1}$. During the first 4 steps the pH (6.9 to 7.2) and

Table 4. Operational conditions and reactor efficiency during the study on the shortening of the HRT

Parameter	**1**	**2**	**3**	**4**	**5**
HRT (h)	24.0	23.0	21.0	19.7	17.4
Substrate COD ($mg.L^{-1}$)	8 386	9 077	7 630	8 003	7 938
Substrate pH	6.0	6.0	6.0	6.0	6.0
Bioreactor effluent pH	6.9	7.0	7.2	7.0	6.4
COD removal (%)	86	89	86	84	79
OLR ($kg\ COD.m^{-3}.d^{-1}$)	8.39	9.47	8.72	9.75	10.95
Alkalinity ($mg.L^{-1}\ CaCO_3$)	1 738	1 750	1 650	1 600	675
Biogas ($L.d^{-1}$)#	7.55	8.16	8.55	9.29	-*
Y_{biogas} ($m^3.kg^{-1}.COD_{removed}$)	0.455	0.421	0.496	0.493	-*

* malfunctioning of biogas measuring unit as a result of excess granules being washed out

methane content of the biogas was 35% CH_4 on average throughout the study

alkalinity (1 650 to 1 750 mg $CO_3.L^{-1}$) of the bioreactor effluent remained fairly constant although re-setting the HRT at 19.7, led to some granule wash-out. Sufficient alkalinity (>1 000 mg $CaCO_3.L^{-1}$) maintaining the methanogenic and non-methanogenic bacteria in a state of equilibrium was most probably the key to satisfactory digestion with COD removal efficiencies of 84 to 89% (Tchobanoglous & Burton, 1991).

The reduction of the HRT from 19.7 to 17.4 led to a considerable decrease in pH and alkalinity. The effluent pH dropped to 6.4 on the first day and to 5.5 by the second day of feeding at an HRT of 17.4 h. The alkalinity level was greatly reduced to 675 mg $CO_3.L^{-1}$ and the COD removal efficiency dropped to 79%. Granule wash-out now also became more severe and led to blockages in the reactor tubes. Blockages led to substantial over-flow of the reactor and subsequent granule loss as well as to the malfunctioning of the gas manometric unit. All attempts to stabilise the reactor at a HRT of 17.4 h failed.

The lowest efficient operational HRT was thus found to be around 19 h with a corresponding OLR of 9.75 kg $COD.m^{-3}d^{-1}$ (Table 4). This HRT was not as short as the HRT of 2.3 h achieved by Torkian & Hashemian (2003) who treated a slaughterhouse wastewater at an OLR of 30 kg $COD.m^{-3}d^{-1}$ in a 1 000 L UASB bioreactor over a 136 d trial period. The reactor system was previously adjusted to slaughterhouse wastewater. The HRT was also not as short as the 10 h achieved by Trnovec & Britz (1998) when treating a cannery wastewater at an OLR of 10.75 $kg.m^{-3}d^{-1}$. It is important to remember that slaughterhouse wastewater has a high protein concentration while cannery wastewater has a high carbohydrate concentration. Cellar wastewater is however, known to be nutrient deficient and is therefore less biodegradable (Toffelmire, 1972).

Conclusions

It is well known that cellar wastewaters are nutrient deficient substrates and are difficult to treat (Toffelmire, 1972). In this study it was shown that an UASB bioreactor could efficiently be used to degrade this type of wastewater. The stabilisation process was, however, lengthy and was probably influenced by too high substrate pH.

The lowest efficient substrate pH was found to be 5.73 for a substrate with COD less than 5 000 $mg.L^{-1}$. Higher substrate loads at this pH led to great decreases in the effluent pH and alkalinity. At a substrate pH of 6.0, it was possible to successfully treat wastewaters (COD removal = 84%) at a HRT of 19.7 h and a corresponding OLR of 9.75 kg $COD.m^{-3}d^{-1}$ producing an effluent with COD of 1 200 $mg.L^{-1}$. Although legal limits of 75 $mg.L^{-1}$ and 400 $mg.L^{-1}$ for disposal in a natural resource and irrigation, respectively (Anon, 1999), were not met, this have great implication for the wine producing industry. The next step would be to investigate the use of AD in combination with a pre- and/or post-treatment.

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CHAPTER 4

INFLUENCE OF OZONATED CELLAR WASTEWATER ON THE GROWTH OF CELLAR EFFLUENT ISOLATES

Summary

The effect of an ozonation treatment of cellar wastewater on growth of bacteria was monitored. Two wastewater batches, from the non-vintage (batch A) and the vintage period (batch B), were used. From both batches growth-substrates for bacterial inoculation, which included the following, were prepared: controls, CON(A) and CON(B); substrates supplemented with nutrients, NUT(A) and NUT (B); and ozonated substrates, OZ(A) and OZ(B). Three strains of naturally occurring bacteria, isolated from raw cellar wastewater, were used as inoculants for the prepared substrates that were incubated at 35°C. The controls supported bacterial growth. In the case of wastewater batch A, the addition of nutrients led to the exponential-phases being lengthened before the stationary-phases were reached from 6 h for the control to 12 h for *Acinetobacter* haemolyticus and at least 24 h for *Burkholderia cepacia* and *Cryseomonas luteola* in substrate NUT(A). However, the maximum log colony forming units (cfu) values were higher in the substrates with added nutrients. The opposite was observed for the substrates prepared from batch B. Ozonation of the wastewater batches for 10 min at a concentration of 73 $mg.L^{-1}$ led to increased growth of the inoculants in substrate OZ(B). For substrate OZ(A), ozonation had an inhibitory effect on growth and it was even detrimental to the growth of *A. haemolyticus*.

Introduction

The wine industry significantly contributes to water intake and subsequent pollution of the water as well the environment (Water Research Commission, 1993). Cellar wastewater is known to exhibit large variations in composition and acidity due to the diversity of activities in the cellar during both the vintage

(pressing, fermentation and maturation) and non-vintage (bottling and cleaning operations) seasons (Bezuidenhout *et al.*, 2002). Strict legislation has forced this industry to implement water management strategies, including wastewater treatment technologies (Hayward *et al.*, 2000). Although anaerobic digestion has proven to be efficient, as with most other biological processes, treatment is often not sufficient to reach the set final legal standard of 75 $mg.L^{-1}$ COD for disposal into a natural water resource (Anon., 1999; Gottschalk *et al.*, 2000).

A chemical oxidation process, such as ozonation, may be of use to improve the biodegradability of compounds in these types of wastewaters as a pre-treatment to biological processes. Martin *et al.* (2002) investigated ozonation as a pre-treatment combined with anaerobic digestion using vinasse as substrate. The function of the ozonation process was to convert refractory and toxic compounds, such as phenol, into simpler molecules of lower molecular mass that could be used as substrate by the anaerobic microbial populations. In this case, ozonation for 2 h decreased the chemical oxygen demand (COD) of the vinasse from 109 200 to 82 100 $mg.L^{-1}$ and led to a small increase in mean specific rate of methane production from 3.56 to 3.67 mL $CH_4.g^{-1}VSS.h^{-1}$. In contrast, Andreozzi *et al.* (1998) found that olive oil mill effluents exposed to an ozonation treatment had an inhibitory effect on methanogenic bacteria in anaerobic digesters due to the formation of inhibitory compounds during ozonation. No inhibitory effect was observed for acidogenic bacteria.

Knowledge and subsequent enhancement of the natural microbial community in a wastewater could possibly be utilised to shorten the start-up period and overall efficiency of the anaerobic digestion process (Van der Merwe & Britz, 1994). Similarly it has been shown that the inoculation of the substrate with specific bacteria (Keyser *et al.*, 2003) or moulds (Jiménez *et al.*, 2003) improved the anaerobic digestion process. In the latter case the substrate was pre-digested with various moulds under aerobic conditions. The pre-digestion led to the breakdown of compounds inhibitory to anaerobic digestion and thus improved biodegradability. Keyser *et al.* (2003) also found that when an *Enterobacter sakazakii* strain isolated from cellar wastewaters was incorporated into upflow anaerobic sludge blanket (UASB) granules, it increased the specificity of the granules to degradation of the cellar wastewater.

The aim of this study was to investigate the possibility of using ozonation to improve the biodegradability or lessen the toxicity in cellar wastewaters for anaerobic treatment. Dominant bacteria present in raw cellar wastewater will be isolated and identified. Accordingly, the growth of these strains will be monitored in substrates that had been exposed to, as well as substrates that had received no ozonation treatments. To eliminate the possibility that nutrient deficiencies may limit bacterial growth, substrates supplemented with an excess of nutrients will also included in the study.

Materials and methods

Wastewater

Batches of non-vintage and vintage-period cellar wastewaters were obtained from the Rupert and Rothschild Fredericksburg cellar (Simondium, South Africa). The substrates used in this study were labelled batch A (non-vintage) and batch B (vintage). The pH, total suspended solids (TSS) and total volatile suspended solids (VSS) of the wastewater batches were determined according to standard methods (APHA, 1992). Orthophosphate phosphorous and COD were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1992).

Microbial isolations

The dominant bacteria in cellar wastewater were isolated and identified as *Acinetobacter haemolyticus*, *Burkholderia cepacia* and *Cryseomonas luteola* according to the methods described in Chapter 3 of this thesis. To isolate these organisms a cellar effluent medium (CE-medium) was used to prepare dilution series of cellar wastewater and pour plates were prepared using CE-medium containing 14 $g.L^{-1}$ agar (Merck). After aerobic and anaerobic incubation at 35°C for 48 h, prevalent strains were inoculated into sterile CE-medium and again incubated at 35°C for 48 h. The dominant bacterial strains were cultivated by successive dilution in CE-medium and the growth phases studied over 14 d. Pure colonies were obtained by repeatedly streaking out selected colonies on CE-agar.

These isolates were then identified using bright field microscopy and API E and NE techniques.

Substrate preparation

All the substrates used for bacterial inoculation had a COD of *ca.* 2 000 $mg.L^{-1}$ and were prepared, as summarised in Table 1, for diluted cellar wastewater batches A and B. The substrate control (CON) received no further treatment. One set of substrates was supplemented with nutrients (NUT) and another exposed to an ozonation treatment (OZ). The pH of all the substrates was adjusted to 7.0 using 1 M KOH prior to being autoclaved (15 min at 121°C and 100 kPa).

Ozonation treatments were done in a glass bubbling column set-up where ozone was bubbled upwards through the column for 10 min. The ozone generator (Parc Scientific, Ifafi) produced ozone at a concentration of 73 $mg.L^{-1}$ as determined by the Iodometric Method (APHA, 1992), at a flow rate of 4 $L.min^{-1}$.

The effect of autoclaving on the chemical composition of the substrates had to be taken into consideration as heat treatment could lead to the breakdown of constituents in the wastewater and subsequently impact the microbial growth. For this reason the inoculation study was also conducted on two non-autoclaved substrates (Table 1, Figure 1). One substrate, OZ(A)NUT, received the same nutrients as substrates NUT(A) and NUT(B) and was exposed to the ozone treatment. Substrate OZ(A)CON was also only exposed to the ozone treatment. Neither of these substrates was autoclaved so as to eliminate any effect the autoclaving process could have on the composition of the substrate.

Growth study

The individual bacterial isolates cultivated on CE-agar were individually inoculated into 20 mL filtered (Whatman no. 1) sterile cellar effluent with a pH adjusted to 7.0 with 1 M KOH and grown for 48 h at 35°C. The inoculated effluent was then centrifuged (Beckman Coulter TJ 25) at 6 000 G for 15 min. The supernatant was discarded and the pellet washed with a 1 mL sterile saline solution (0.85% m/v) and again centrifuged. The supernatant was once more discarded and the pellet used to prepare McFarland 1 (3×10^8 $cfu.mL^{-1}$) suspensions of each of the individual bacteria in sterile saline solution

Table 1. Substrates used in microbial growth evaluation studies

Substrate	Code*	Characteristics
BATCH A		
Control (batch A)	CON(A)	COD = *ca.* 2 000 mg.L^{-1}
Nutrient supplemented (batch A)	NUT(A)	COD = *ca.* 2 000 mg.L^{-1} Nutrients: 250 mg.L^{-1} urea 170 mg.L^{-1} K_2HPO_4 250 mg.L^{-1} $KHCO_3$ 250 mg.L^{-1} sucrose
Ozonated (batch A) Ozonated for 10 min	OZ(A)	COD = *ca.* 2 000 mg.L^{-1}
Ozonated (batch A) control (not autoclaved)	OZ(A)CON	COD = *ca.* 2 000 mg.L^{-1}
Ozonated (batch A) nutrient supplemented (not autoclaved)	OZ(A)NUT	COD = *ca.* 2 000 mg.L^{-1} Nutrients: 250 mg.L^{-1} urea 170 mg.L^{-1} K_2HPO_4 250 mg.L^{-1} $KHCO_3$ 250 mg.L^{-1} sucrose
BATCH B		
Control (batch B)	CON(B)	COD = *ca.* 2 000 mg.L^{-1}
Nutrient supplemented (batch B)	NUT(B)	COD = *ca.* 2 000 mg.L^{-1} Nutrients: 250 mg.L^{-1} urea 170 mg.L^{-1} K_2HPO_4 250 mg.L^{-1} $KHCO_3$ 250 mg.L^{-1} sucrose
Ozonated (batch B) Ozonated for 10 min	OZ(B)	COD = *ca.* 2 000 mg.L^{-1}

* A = Cellar wastewater batch A
B = Cellar wastewater batch B
CON = Control
NUT = Nutrient supplemented
OZ = Ozonated

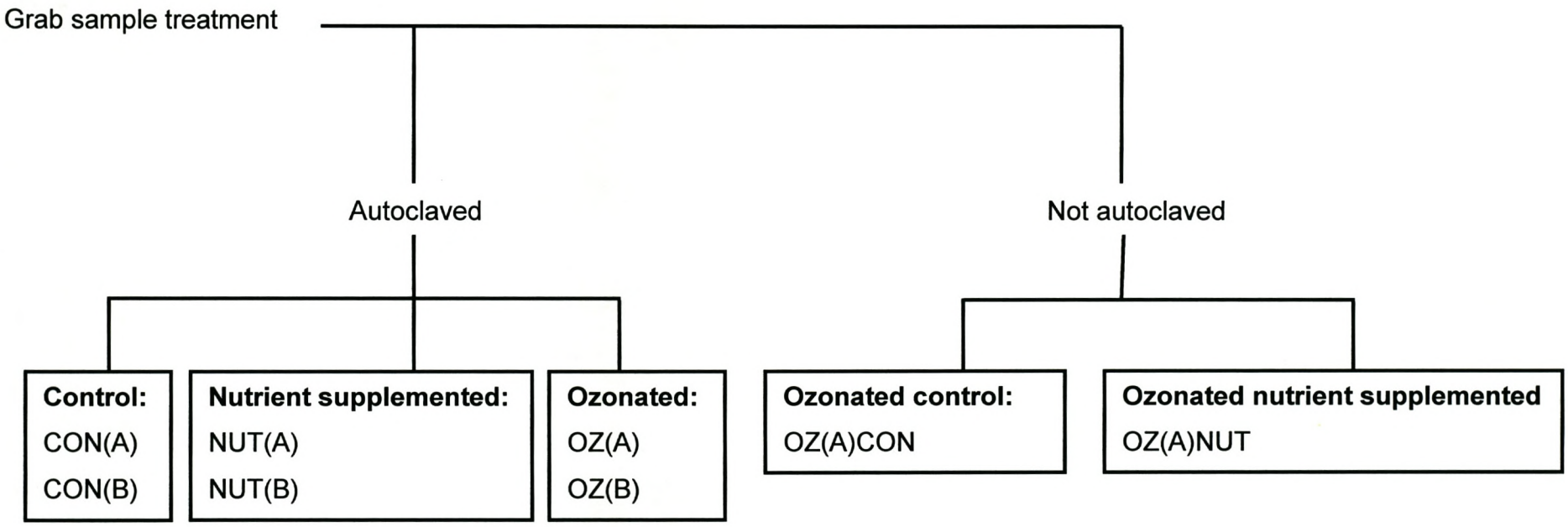

Figure 1. Diagrammatic representation of substrate preparation for microbial growth evaluation studies.

(0.85% m/v). The different cellar wastewater substrates (Table 1) were then inoculated with 1% (v/v) of the various bacterial suspensions. The inoculated substrates were incubated at 35°C and bacterial growth was monitored over 24 h. Enumeration was done by dilution series of the different substrates prior to inoculation (t = 0-1), just after inoculation (t = 0), and then every 6 h. The plate count agar (PCA) plates were incubated for 48 h at 35°C. The growth study, using the same wastewater batches for substrate preparation, was performed in duplicate.

Results and discussion

Wastewater

The compositions of the two wastewater grab-sample batches are summarised in Table 2. It is evident that the two batches (A and B) showed considerable differences in organic content, which was measured as COD (3 520 and 10 202 $mg.L^{-1}$) and pH (6.3 and 3.6), respectively. This was expected since cellar wastewater varies according to cellar operation during vintage and non-vintage periods (Van Schoor, 2000).

Growth study

No growth was detected before inoculation (t = 0-1) on the CON(A), CON(B), NUT(A), NUT(B), OZ(A) or OZ(B) plates. After inoculation (t = 0) and incubation all isolates were detected as white, round colonies in or on the PCA plates.

Inoculation of substrate CON(A), led to substantial growth of *Acinetobacter haemolyticus* from 4.9 to 6.4 log $cfu.mL^{-1}$, *Burkholderia cepacia* from 5.5 to 6.8 log $cfu.mL^{-1}$ and *Cryseomonas luteola* from 5.2 to 6.5 log $cfu.mL^{-1}$, during the first 6 h of incubation, respectively (Fig. 2). After 6 h, the stationary phases for all the species in CON(A) had been reached with the maximum log $cfu.mL^{-1}$ during the 24 h incubation period being 6.8, 7.3 and 7.4, for the respective strains.

The microbial cell concentration in the inoculums used for the inoculation of substrate CON(B) were lower than that used as inoculum for substrate CON(A).

Table 2. Compositions of the two wastewater grab-sample batches, obtained from the Rupert and Rothschild Fredericksburg cellar

Parameter	Grab-sample	
	A	B
COD_{total} ($mg.L^{-1}$)	3 533 (±SD 865)	10 202 (±SD 794)
$COD_{soluble}$ ($mg.L^{-1}$)	2 690	9 936
pH	6.3	3.6
Phosphorous (as mg $PO_4.L^{-1}$)	20	39
TSS ($mg.L^{-1}$)	648	572
VSS ($mg.L^{-1}$)	562	507

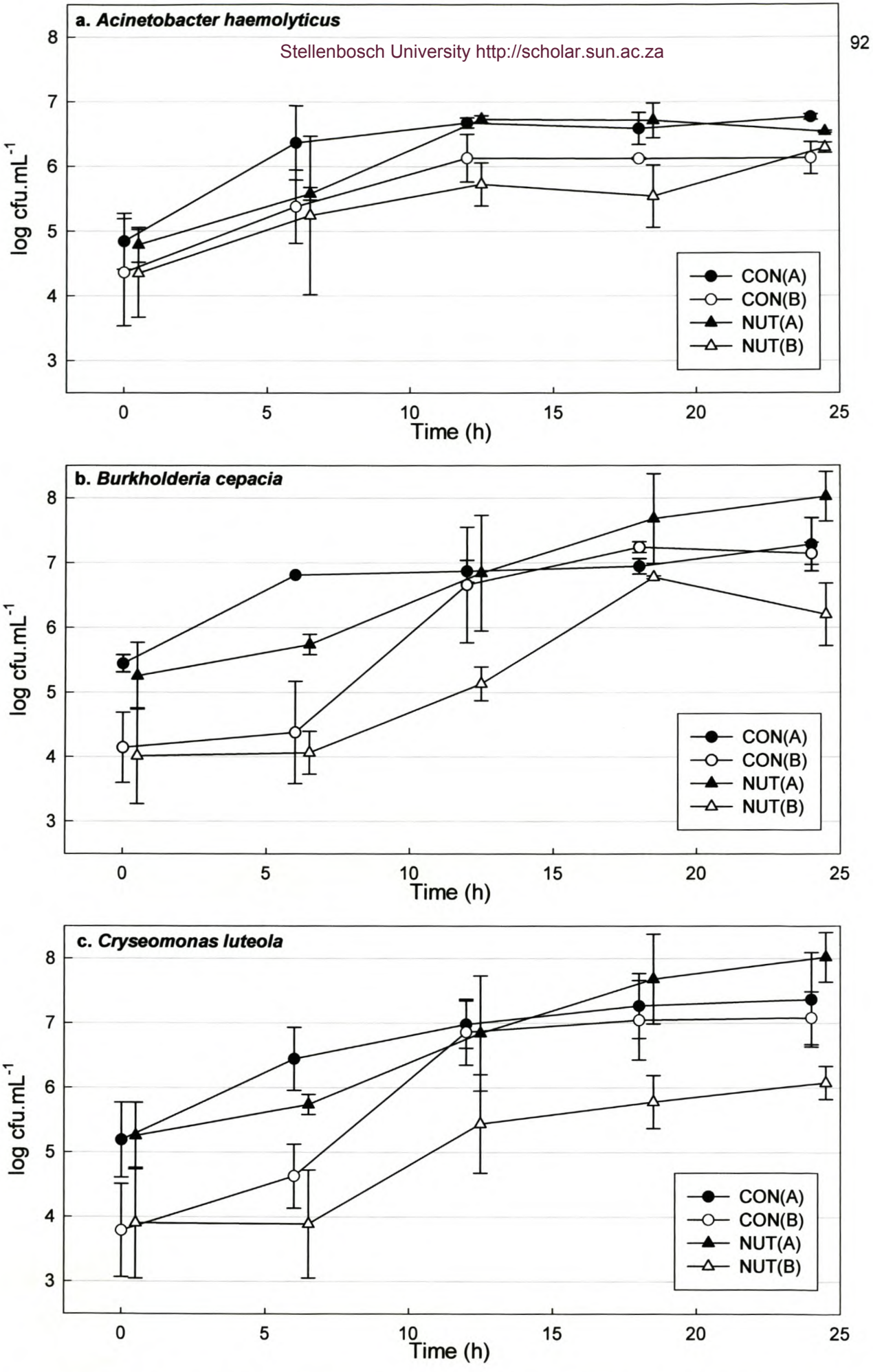

Figure 2. Growth of a. *Acinetobacter haemolyticus*, b. *Burkholderia cepacia* and c. *Cryseomonas luteola* in the CON and NUT substrates.

Although a McFarland 1 standard was used to standardize the cell concentration in the inoculums, cellar wastewater particles not removed by filtration could have had an influence on the turbidity of the suspension. The lower amount of cells could also have led to the longer exponential-phases of at most 12 h in CON(B). Differences in substrate composition of CON(A) and CON(B) could also have led to the extension. However, the stationary phases for *B. cepacia* and *C. luteola* reached the same log cfu.mL^{-1} concentrations as those in CON(A) (Fig. 2b and c). For *A. haemolyticus*, the maximum log cfu.mL^{-1} of 6.1 in the stationary phase, was lower in CON(B) than in CON(A) (Fig. 2a).

The growth curves of the *B. cepacia* and *C. luteola* strains in NUT(A) (Fig. 2), where the substrate had been enhanced with nutrients, showed a longer exponential-phase than in CON(A) (Fig. 2). For *B. cepacia* and *C. luteola* the stationary-phases were still not reached even after 24 h incubation at 35°C. The maximum log cfu.mL^{-1} concentrations reached were 7.9 and 8.0 for *B. cepacia* and *C. luteola*, respectively. In these cases it appears as though the added nutrients led to an increase in growth when compared to growth in CON(B). For *A. haemolyticus* the time from the exponential-phase to the stationary-phase was only 12 h and the maximum cell number reached was 6.7 log cfu.mL^{-1}.

As in the case of the inoculums in substrate CON (A and B), the inoculums in substrate NUT(B) were again lower than in substrate NUT(A) (Fig. 2). The maximum log cfu.mL^{-1} reached were 6.3, 6.8 and 6.1 for *A. haemolyticus*, *B. cepacia* and *C. luteola*, respectively. When the growth of the three bacterial strains in the various substrates prepared from wastewater batch B are compared, it appears as though the added nutrients in substrate NUT may have been inhibitory (Fig. 2 and 3). The bacteria possibly had become acclimatised to an energy poor environment (carbohydrate deficient).

Substrate OZ(A), which received the ozone treatment, showed inhibition to all three cultures (Fig. 4). In the case of *A. haemolyticus*, the substrate had a detrimental effect with the cell number being lowered from 4.9 to 3.4 log cfu.mL^{-1} by the 24 h incubation period (Fig. 4a). The possibility of wastewater batch A containing a substance that could form an inhibitory compound after ozonation, must be considered. Previously Andreozzi *et al.* (1998) also reported that olive oil mill effluents exposed to an ozonation treatment had an inhibitory effect on methanogens due to the formation of inhibitory

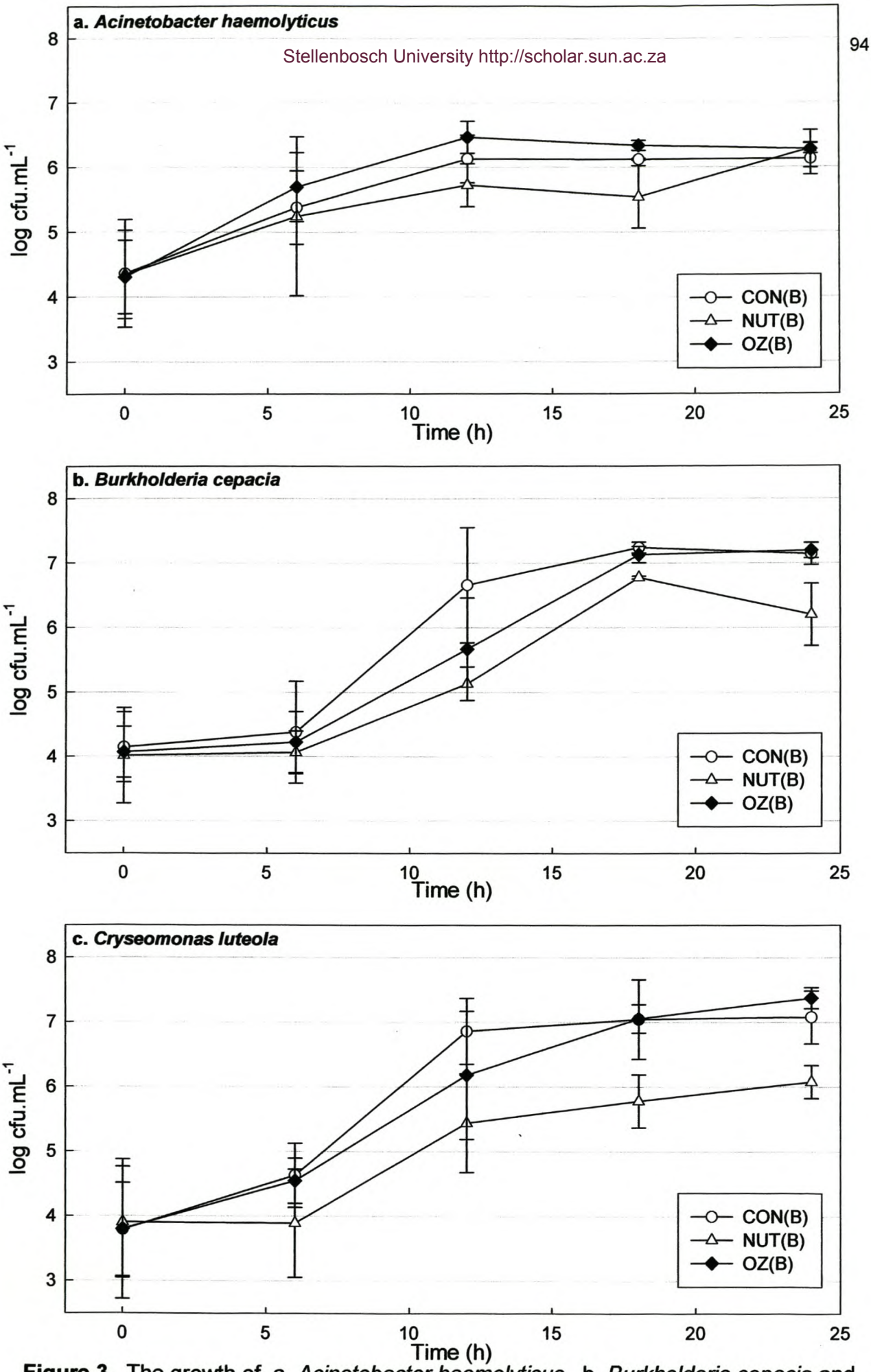

Figure 3. The growth of a. *Acinetobacter haemolyticus*, b. *Burkholderia cepacia* and c. *Cryseomonas luteola* in substrates prepared from wastewater batch B: CON(B); NUT(B); and OZ(B).

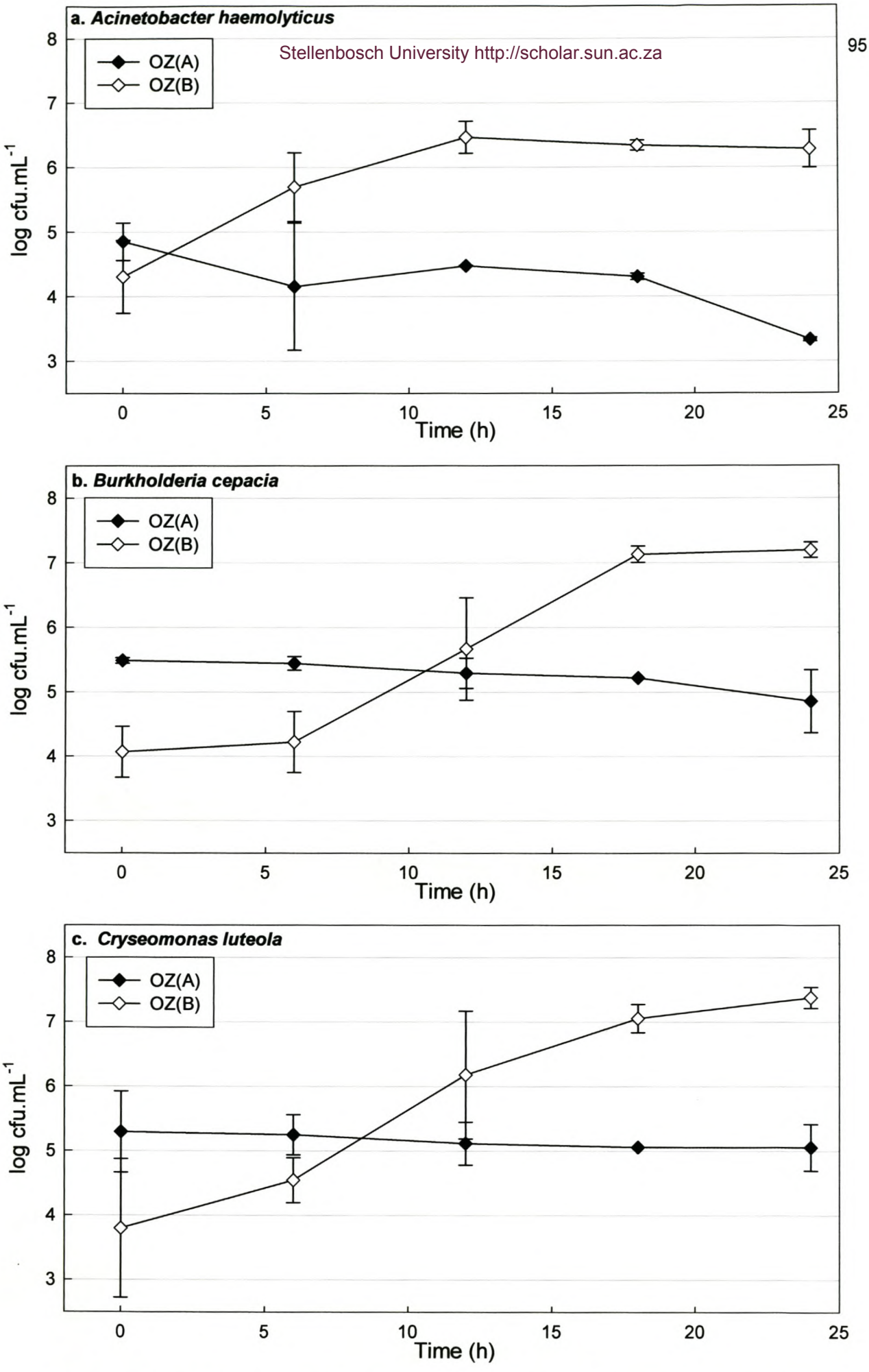

Figure 4. Growth of a. *Acinetobacter haemolyticus*, b. *Burkholderia cepacia* and c. *Cryseomonas luteola* in the OZ substrates.

compounds during ozonation. However, in their case no inhibitory effect was observed for acidogenic bacteria. The effect of an ozone treatment on pure culture bacteria isolated from the substrate itself and then inoculated into the ozonated substrates were not investigated by them.

In comparison with substrate OZ(A) that was detrimental to bacterial growth, substrate OZ(B) showed growth enhancement (Fig. 3). The exponential-phase to stationary-phase for *A. haemolyticus* was reached after 12 h and for *B. cepacia* and *C. luteola* it was reached after 18 h. Although the exponential-phases for the isolates *B. cepacia* and *C. luteola* were longer than the exponential-phases for the same isolates in substrate CON(B), the maximum log cfu.mL^{-1} at maximum growth were slightly higher, reaching 7.3 and 7.4 for these two isolates (Fig. 3). It was thus concluded that ozonation played a role in increased growth and thus possibly also improved the biodegradability of substrate OZ(B).

The possibility of the "inhibitory compound" found in substrate OZ(A) only being inhibitory after autoclaving the substrate was also investigated. Substrates prepared for this study were not autoclaved and although exposed to ozone, which is well known for its disinfectant abilities (Greenwood & Earnshaw, 1984; Rice, 2001), the substrates were not sterile (t = 0-1). Substrate OZ(A)CON and OZ(A)NUT had cfu.mL^{-1} values (average of three substrates prepared for inoculation with three isolates) of 5.8 x 10^3 and 1.5 x 10^4 , respectively, prior to inoculation (t = 0-1). In substrate OZ(A)CON there was no increase in the numbers of any of the inoculums plus the microbial population, which was still present in the substrate after ozonation (Fig. 5a). As was the case in the autoclaved substrate, OZ(A), a decrease in viable count was marked in the substrate inoculated with *A. haemolyticus*.

A similar trend to what was observed for OZ(A)CON was also observed for OZ(A)NUT (Fig. 5b). In this case the viable count for all three cultures was lower after 24 h of incubation than right after inoculation. The theory that autoclaving could lead to the formation of inhibitory compounds in ozonated substrates was rejected. It was concluded that the "inhibitory compound" was probably formed by the ozonation process.

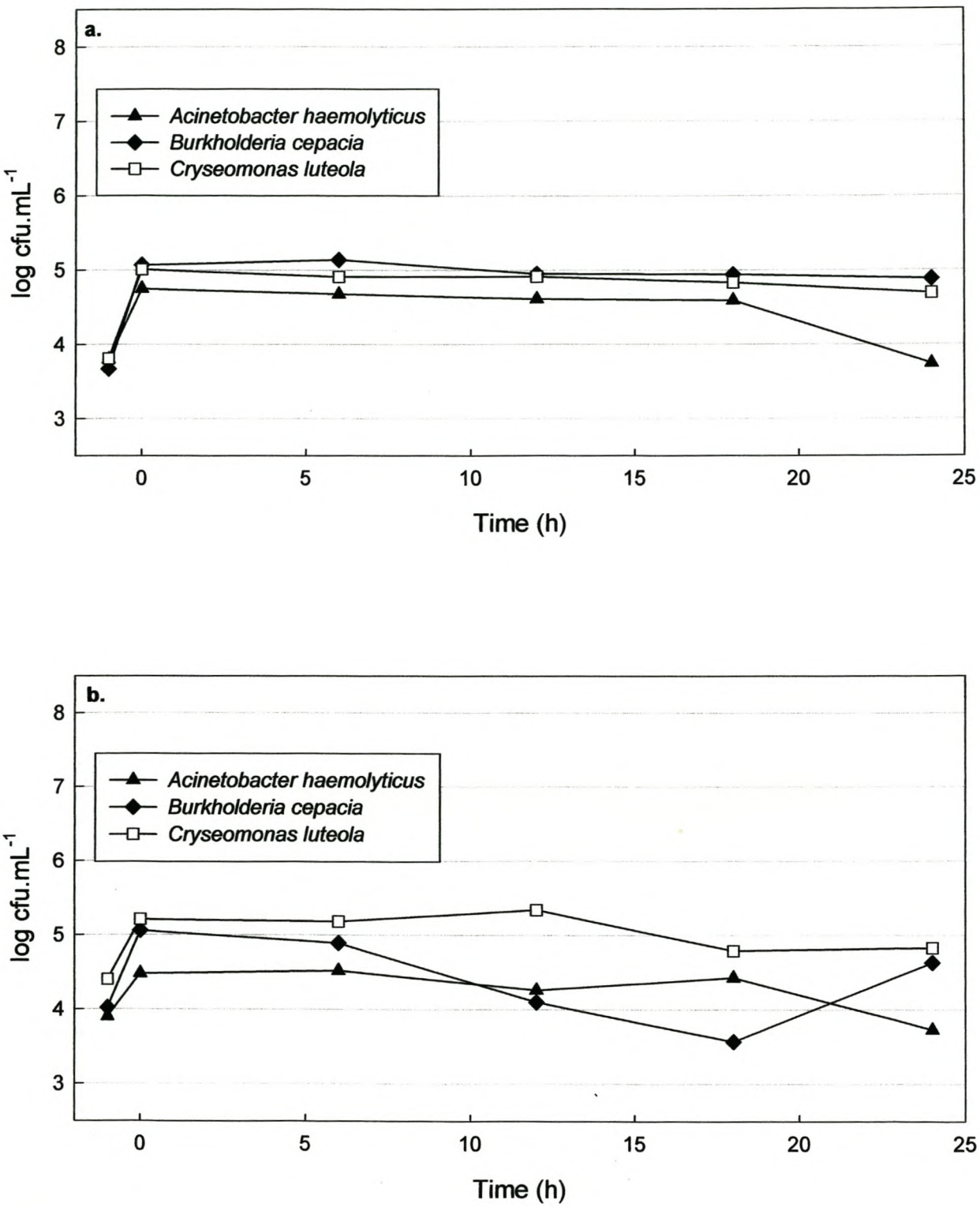

Figure 5. The growth of cellar effluent isolates in ozonated cellar wastewater substrate not exposed to an autoclaving process: a. OZ(A)CON and b. OZ(A)NUT.

Conclusions

From the data obtained in this study cellar wastewater can generally be considered as a good growth medium for the natural bacteria isolated from raw cellar wastewaters. However, the composition of the wastewater varies according to cellar operation and seasonal activity and the subsequent composition affects the bacterial growth. The data also showed that the addition of nutrients enhanced the growth of bacterial isolates in a cellar wastewater substrate from the non-vintage season (wastewater batch A). It was also found that the addition of the same concentration of nutrients to a cellar wastewater from the vintage period led to lower growth than in the control substrate from the same wastewater batch (Batch B). In the case of ozone treatment a slight enhancement of the bacterial growth in substrate batch B (vintage) was observed. The same treatment was inhibitory and even detrimental to growth in substrate batch A.

In conclusion the results of this study showed that ozonation could be used to improve biodegradability of cellar wastewaters. However, it is important to evaluate the effectiveness of ozone treatment for each cellar wastewater batch as it can easily lead to the substrate becoming inhibitory. A detailed description of the cellar wastewater composition and the products formed during ozonation might lead to a better understanding of the "inhibitory phenomenon".

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CHAPTER 5

EFFICIENCY OF OZONATION IN THE PRE- AND POST-TREATMENT OF UASB TREATED CELLAR WASTEWATERS

Summary

The efficiency of ozonation as a pre-, post- and combined pre- and post-treatment to upflow anaerobic sludge blanket (UASB) treatment was investigated. A 5 min ozonation treatment process at a concentration of 73 $mg.L^{-1}$ was found to be optimal for both a pre- and post-treatment. Ozonation of cellar wastewater led to a 20% reduction in chemical oxygen demand (COD). The total suspended solids (TSS) and the volatile suspended solids (VSS) were both reduced by 73%. Polyphenol concentration was reduced by more than 73%. The UASB treatment alone led to a COD reduction of 67 to 85%, depending on the specific cellar wastewater. The TSS and VSS concentrations were reduced by 80 and 81%, respectively. The COD, TSS and VSS reduction could be increased to 88, 97 and 98%, respectively, when UASB treatment was followed by a post-ozonation treatment. Ozonation also led to an 80% reduction in colour of the UASB reactor effluent. Pre-ozonation of the cellar wastewater led to a substantial increase in COD removal to 86% for an UASB reactor with a previous maximum COD removal efficiency of 67%. Biogas production also increased from 1.4 $L.d^{-1}$ to 3.8 $L.d^{-1}$. Both the TSS and VSS reduction for a pre-ozonation UASB combined process, were 95%. Further post-ozonation led to the COD removal efficiency being increased to 89% and a total reduction of 99% for both TSS and VSS.

Introduction

The wine industry contributes greatly to pollution of a limited natural resource, water (Van Schoor, 2000). The wastewaters, which originate from cellar cooling operations and the washing of floors, vessels and equipment (Water Research Commission, 1993; Bezuidenhout *et al.*, 2002), are nutrient deficient and have a

tendency of becoming acidic and odorous when left standing (Toffelmire, 1972). Cellar wastewater has a chemical oxygen demand (COD) of 800 to 12 800 $mg.L^{-1}$ (Petruccioli *et al.*, 2002) depending on the season and precise cellar activities.

As wine production has increased during the last decade, the pressure on natural resources, specifically water, has also increased. Public concern for the environment, high levies charged for municipal treatment of wastewater and the aspiration to stay competitive in an increasingly globalised market place (Hayward *et al.*, 2000), have compelled cellars to consider on-site wastewater treatment options.

Anaerobic digestion (AD), specifically utilising upflow anaerobic sludge blanket (UASB) technology, has been shown to be feasible to treat various food and beverage processing wastewaters, including wastewaters from wine cellars (Ross, 1989; Strydom *et al.*, 1997; Puñal & Lema, 1999; Ronquest & Britz, 1999; Sigge *et al.*, 2002). Müller (1998) found removal efficiencies for COD and BOD of over 90% when treating a cellar wastewater in an UASB reactor with a 50 m^3 volume at influent COD levels of 941 to 13 600 $mg.L^{-1}$. In another study a COD removal of more than 93% at an organic loading rate (OLR) of 10.1 kg $COD.m^{-3}d^{-1}$, a hydraulic retention time (HRT) of 14 h and an influent pH of 5.1, was achieved with a laboratory UASB system (Ronquest & Britz, 1999). Under these conditions the final reactor effluent COD was 182 $mg.L^{-1}$. The effluent COD was still above the legal limit of 75 $mg.L^{-1}$ for discharge in a natural water system (Anon., 1999). To reduce the effluent COD to below legal limits, a further polishing step would be necessary.

Post-ozonation has been shown to be efficient as a polishing step. Athanasopoulos & Athanasopoulos (1998) used an UASB reactor to reduce the COD of "currant" wastewater by 95.5%, yielding an effluent with a COD of only 185 $mg.L^{-1}$. Ozonation of the effluent for 3 h at a concentration of 300 $mg.h^{-1}$ led to the COD being further reduced to below 50 $mg.L^{-1}$. Similarly, Sigge *et al.* (2002) found UASB treatment to lower the COD of cannery and cellar wastewaters by 93 and 96%, respectively. Post-ozonation for 30 min using a granular activated carbon column led to further COD reductions of the effluents by as much as 53 and 55% for cannery and cellar wastewaters, respectively. The lowest COD achieved was 247 $mg.L^{-1}$ for the cannery wastewater and 67 $mg.L^{-1}$ for the cellar

wastewater, representing total COD reductions of more than 98% for both wastewaters.

The AD process is often limited by compounds in the wastewater not being biodegradable (Gottschalk *et al.*, 2000). It is known that chemical oxidation methods, such as ozonation as a pre-treatment may convert refractory and toxic compounds into simpler molecules with lower molecular weights that could then be used as substrate by the anaerobic populations. Andreozzi *et al.* (1998) showed that total phenols and unsaturated lipids in an olive mill wastewater could be reduced by 50% after a 3 h ozonation process. Martin *et al.* (2002) reported that the COD of vinasse could be decreased from 109 200 $mg.L^{-1}$ to 82 100 $mg.L^{-1}$ (25% decrease) using a 2 h ozonation process. Benitez *et al.* (1999) also found that the COD of a wine distillery wastewater could be lowered by 20% by ozonation alone. When the ozonated substrate replaced the normal substrate of a magnetically stirred anaerobic batch digestion unit, the methane yield coefficient increased from 187 to 215 mL $CH_4.g^{-1}$ COD degraded.

The aim of this study was firstly to investigate the feasibility of the following ozone combined UASB treatments for cellar wastewater degradation: ozone pre-treatment; ozone post-treatment; and an ozone pre- and post-treatment. As part of the study, the performance of an UASB reactor treating pre-ozonated cellar wastewater will be monitored.

Materials and methods

Wastewater

Cellar wastewaters were obtained from the Rupert and Rothschild Fredericksburg (R-RF) (Simondium, South Africa) (March 2003) and the Bergkelder, Distell (BK) (Stellenbosch, South Africa) (August 2003) cellars. The substrate batches were kept at -18°C in 25 L containers. When required, individual containers were defrosted and then stored at 4°C.

Anaerobic treatment set-up

A laboratory-scale UASB bioreactor with an operational volume of 2.3 L (Trnovec & Britz, 1998) was used and operated at 35°C (Meyer *et al.*, 1983). The

upflow velocity was set at 2.8 $m.h^{-1}$ by using a recirculation pump and substrate was pulse-fed from the bottom of the reactor using a peristaltic pump (Watson-Marlow 101U/R) controlled by an electronic timer. The volume of biogas, which exited at the top via an open gas/solid separator, was determined using a manometric unit fitted with a gas-tight valve and an electronic counter. The overflow of the bioreactor drained through a U-shaped tube to prevent atmospheric oxygen entering the system.

Ozonation set-up

Ozonation proceeded in a continuous mode bubble contacting system. Ozone was bubbled upwards through a glass column for the duration of the ozonation treatment. The bubbling column with a length of 0.90 m and diameter of 0.06 m had a sintered glass disc at one end for bubble generation. An ozone generator (Parc Scientific, Ifafi) capable of producing 28.0 $g.h^{-1}$ ozone was used for the ozonation trials. Ozone concentrations of 47 and 73 $mg.L^{-1}$ were used during the studies. The bubbling column contained 1.70 L of wastewater for both the pre- and post-ozonation studies and the treatments were done at room temperature. The flow rate was kept at 4 $L.min^{-1}$.

Analytical methods

The ozone concentrations were determined using an iodiometric titration technique (APHA, 1992) and the polyphenol concentration in the wastewater was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). The COD, orthophosphate phosphorous (PO_4); and total Kjeldahl nitrogen (TKN) were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1992). Total suspended solids (TSS), volatile suspended solids (VSS) and pH were measured according to standardised procedures (APHA, 1992). Colour removal in the UASB effluent was determined by monitoring the absorbance of the effluent at 288 nm using a spectrophotometer (Spectronic Instruments, USA).

The volatile fatty acid (VFA) concentration of the UASB effluent was determined using a Varian (Model 3700) gas chromatograph, equipped with a flame ionisation detector and a 30 m fused silica capillary column with FFAP bonded stationary phase (Quadrex Co. New Haven). The column temperature

was initially held at 105°C for 2 min, and then increased at a rate of 8°C per min to 190°C. The detector and inlet temperatures were set at 300° and 130°C, respectively, and nitrogen gas was used as carrier gas at a flow rate of 6.1 $mL.min^{-1}$.

A Varian 3300 gas chromatograph equipped with a thermal conductivity detector and 2.0 m x 3.0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh, was used to determine biogas composition. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 30 $mL.min^{-1}$.

Experimental studies

Various combinations of pre- and post-ozonation and UASB treatment were investigated. The removal of COD, TSS, VSS, colour and polyphenols at a range of ozonation times from 1 to 10 min and the optimal treatment time, were determined. A description of the treatment combinations is listed in Table 1.

Treatment A involved an investigation into the effect of different ozone concentrations and ozonation times on cellar wastewater in an attempt to reach the highest possible COD reduction. The BK wastewater was used as substrate for this part of the study. Ozone concentrations of 47 and 73 $mg.L^{-1}$ at a flow-rate of 4 $L.min^{-1}$ were used. The effect of ozonation on the polyphenol content of cellar wastewater was also determined in triplicate. Polyphenol degradation was monitored in both the R-RF and BK wastewater batches.

Treatment B, which only focused on the biological treatment step, namely the UASB activity, and Treatment C (UASB + O_3) were performed on the R-RF wastewater. During these treatment studies the HRT and OLR of the UASB reactor was set at 24 h and 5.4 kg $COD.m^{-3}d^{-1}$, respectively. Substrate pH was adjusted to 6.0 using 1 M KOH and additions of sucrose (500 $mg.L^{-1}$), urea (500 $mg.L^{-1}$) and K_2HPO_4 (200 $mg.L^{-1}$) were made to prevent nutrient limitations. $KHCO_3$ (500 $mg.L^{-1}$) was added to sustain alkalinity. Post-ozonation (Treatment C) involved ozone treatment at a concentration of 73 $mg.L^{-1}$ (4 $L.min^{-1}$) for various times (1, 2, 5, 10 min).

The treatments involving ozonated wastewaters for use as UASB substrates (treatments D and E) were performed on the BK wastewater. During

Table 1. Pre- and post-treatment combinations of cellar wastewaters

Treatment	Description
A	Ozonation of cellar wastewater
B	UASB treatment of cellar wastewater
C	UASB treatment + post-ozonation
D	Pre-ozonation + UASB treatment
E	Pre-ozonation + UASB treatment + post-ozonation

these treatment studies, the HRT and OLR of the UASB reactor were set at 24 h and 4 kg $COD.m^{-3}d^{-1}$, respectively. Previously (Chapter 3 of this thesis), when the reactor was treating cellar wastewater at a HRT of 17.4 h, OLR of 10.95 kg $COD.m^{-3}d^{-1}$ and substrate pH of 6.0, severe granule wash-out and reactor acidification were experienced. Accordingly the HRT was re-adjusted to 24 h and the substrate pH was adjusted to 7.0 at the start of this part of the study and kept constant for the duration of the study. The reactor was acclimatised to a "raw diluted cellar wastewater substrate" with a COD of 4 000 $mg.L^{-1}$ and supplemented with nutrients as in Treatment B. Once the reactor had stabilised, "ozonated substrate" replaced the "raw diluted cellar wastewater substrate".

The ozone pre-treated cellar wastewater substrate was prepared using the bubble column system, as described above. An ozonation time of 5 min was used to treat the undiluted cellar wastewater at an ozone concentration of 73 $mg.L^{-1}$. The pre-ozonated wastewater was also diluted to a COD of 4 000 $mg.L^{-1}$ and the mentioned nutrient additions were made. Treatment E involved a post-ozonation treatment process of the reactor effluent from Treatment D at a concentration of 73 $mg.L^{-1}$ (4 $L.min^{-1}$) for various times (1, 2, 5, 10 min).

Results and discussion

Wastewater

The compositions of the wastewater batches are given in Table 2. Both batches were high in organic content with CODs above 8 000 $mg.L^{-1}$. The wastewater batches had a low nitrogen and phosphorous content necessitating N and P supplementation before use as reactor substrate.

Treatment A

Initial pre-ozonation trials on the BK cellar wastewater (COD = 8 000 $mg.L^{-1}$, pH = 4.5) resulted in COD reductions of up to 20% after a 5 min ozonation at ozone concentrations of 47 and 73 $mg.L^{-1}$ (Fig. 1). No further COD reductions were observed upon extending the ozone treatment time and a 5 min ozone contact time was accepted as best for COD degradation with the

Table 2. Composition of the wastewater batches obtained from the Rupert and Rothschild Fredericksburg (R-RF) and Bergkelder (BK) cellars

Parameter	Batch	
	R-RF	BK
COD ($mg.L^{-1}$)	10 206	8 000
pH	3.6	4.5
Phosphorous (mg $PO_4.L^{-1}$)	39	39
TKN ($mg.L^{-1}$)	6	35
Polyphenols as gallic acid equivalents ($mg.L^{-1}$)	50	74
TSS ($mg.L^{-1}$)	572	1 180
VSS ($mg.L^{-1}$)	507	1 123
C:N:P ratio	1701:1:6.5	228.6:1:1.1

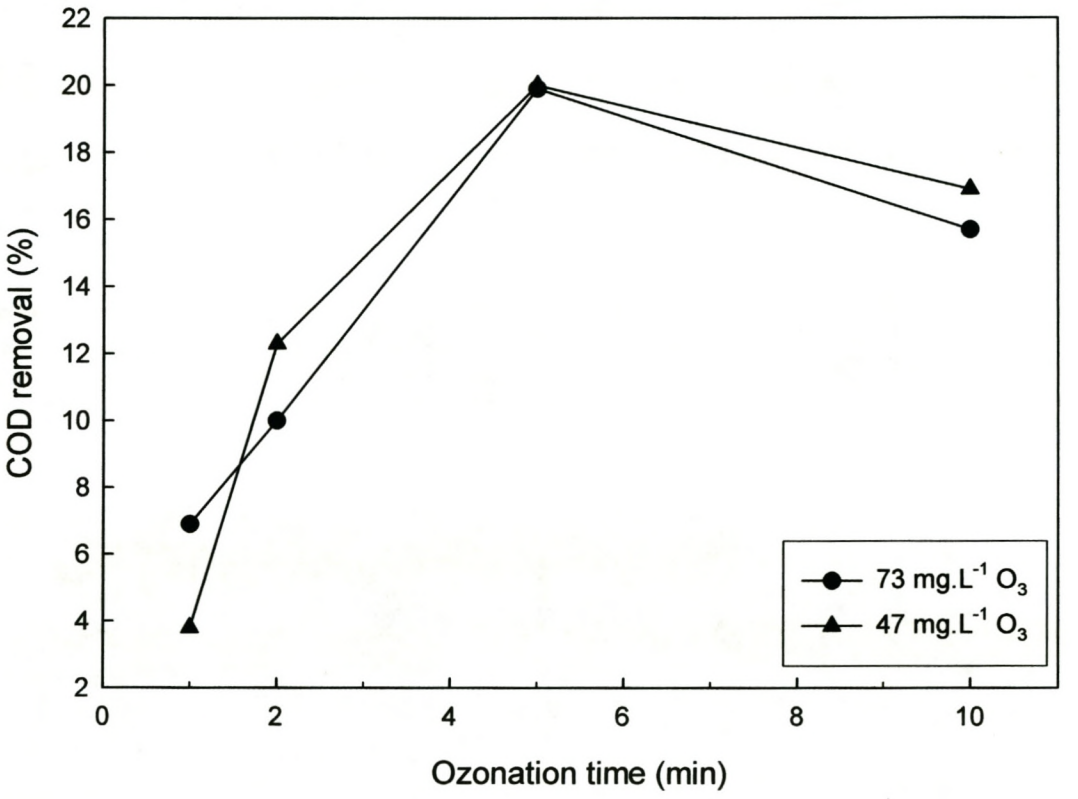

Figure 1. The effect of increasing ozonation time on the COD removal during the treatment of BK cellar wastewater with ozone (Treatment A) (starting COD = 8 000 $mg.L^{-1}$).

experimental set-up used. The COD reductions at the ozone concentrations of 47 and 73 $mg.L^{-1}$ were similar. The results for COD removal were contradictory to those reported by Marais (2001) who did not find any reduction after ozonation in the COD of a cellar wastewater when the cellar wastewater had a pH lower than 7.5. However, the results from this study correlated well with those obtained by Benitez *et al.* (1999) who also achieved a COD reduction of 20% for a distillery wastewater (initial COD = 18 400 $mg.L^{-1}$) at a pH of *ca.* 4.0. Similarly, Sigge *et al.* (2002) found the COD of cellar wastewater (pH = 4.8; COD = 3 700 $mg.L^{-1}$) could be reduced by 30% after a 5 min ozone treatment.

The data also showed that ozonation led to a reduction in the polyphenol concentration of both the R-RF and BK wastewaters (Fig. 2). For the R-RF wastewater the polyphenol concentration was reduced by 80% from 49.8 to 10.2 mg gallic acid equivalents ($mg.L^{-1}$) during the first 2.5 min of the ozonation treatment. For the same treatment time the polyphenol concentration in the BK wastewater was reduced by 73% from 74.3 to 19.8 mg gallic acid equivalents ($mg.L^{-1}$). A further 2.5 min ozonation period resulted in only a slight improvement in polyphenol reduction from 10.2 to 9.8 and 19.8 to 19.3 gallic acid equivalents ($mg.L^{-1}$) for the R-RF and BK wastewaters, respectively. The polyphenol reduction was necessary, as phenolic compounds are known to lead to inhibitory and antibacterial activity in wastewaters during biological treatment. Polyphenols are specifically toxic to methanogenic bacteria and inhibit the production of methane and consequently, the efficiency of the anaerobic digestion process is decreased (Hamdi, 1993).

Treatment B and C

Treatment B involved the treatment of a diluted R-RF wastewater (COD = 5 400 $mg.L^{-1}$, TSS = 303 $mg.L^{-1}$, VSS = 268 $mg.L^{-1}$) using the UASB reactor. During the study the HRT and OLR were kept constant at 24 h and 5.4 kg $COD.m^{-3}.d^{-1}$, respectively. The UASB reactor effluent had a pH > 6.9 and the average reactor COD removal efficiency was 85%. The TSS and VSS were reduced by 80 and 81%, respectively.

Post-ozonation (5 min) of the R-RF wastewater from the above described UASB reactor led to a further 22.7% COD reduction (Fig. 3). An increase in the ozonation time to 10 min only resulted in a slight increase in COD removal to

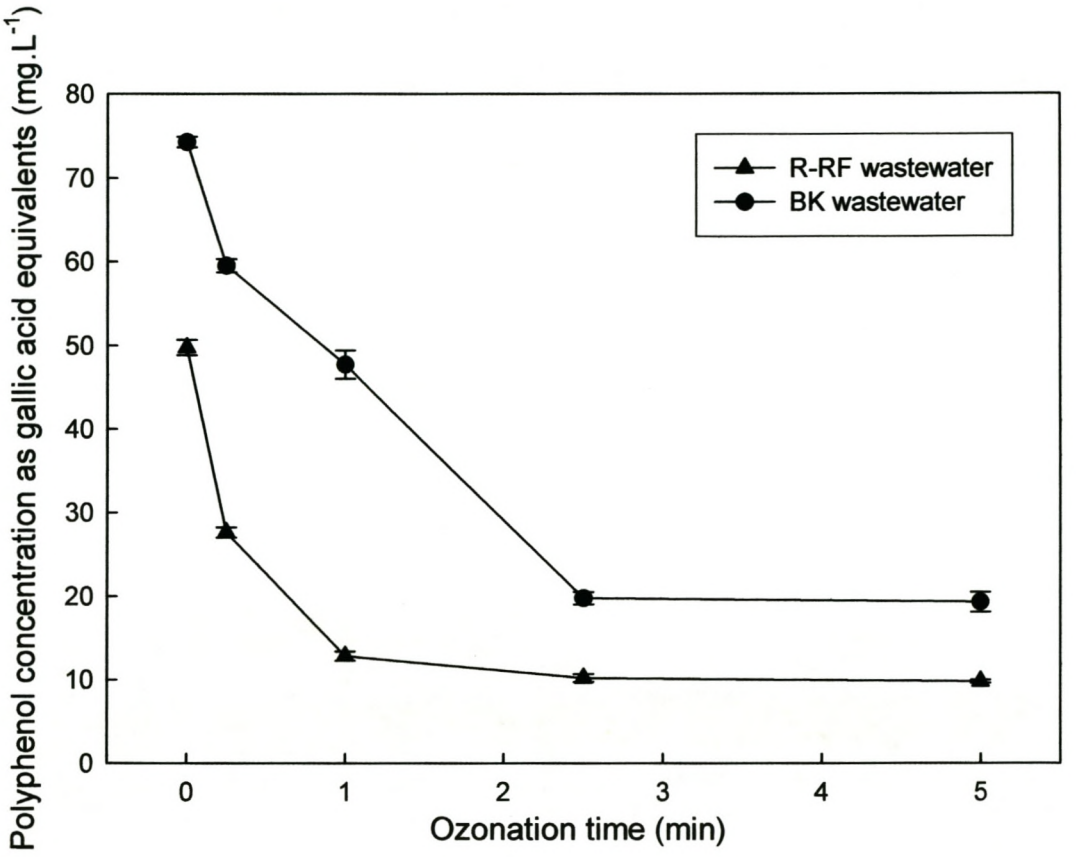

Figure 2. The change in polyphenol concentration in cellar wastewaters when treated with 73 $mg.L^{-1}$ ozone (flow-rate = 4 $L.min^{-1}$) (n = 4) (the standard deviation was used as error-bar length).

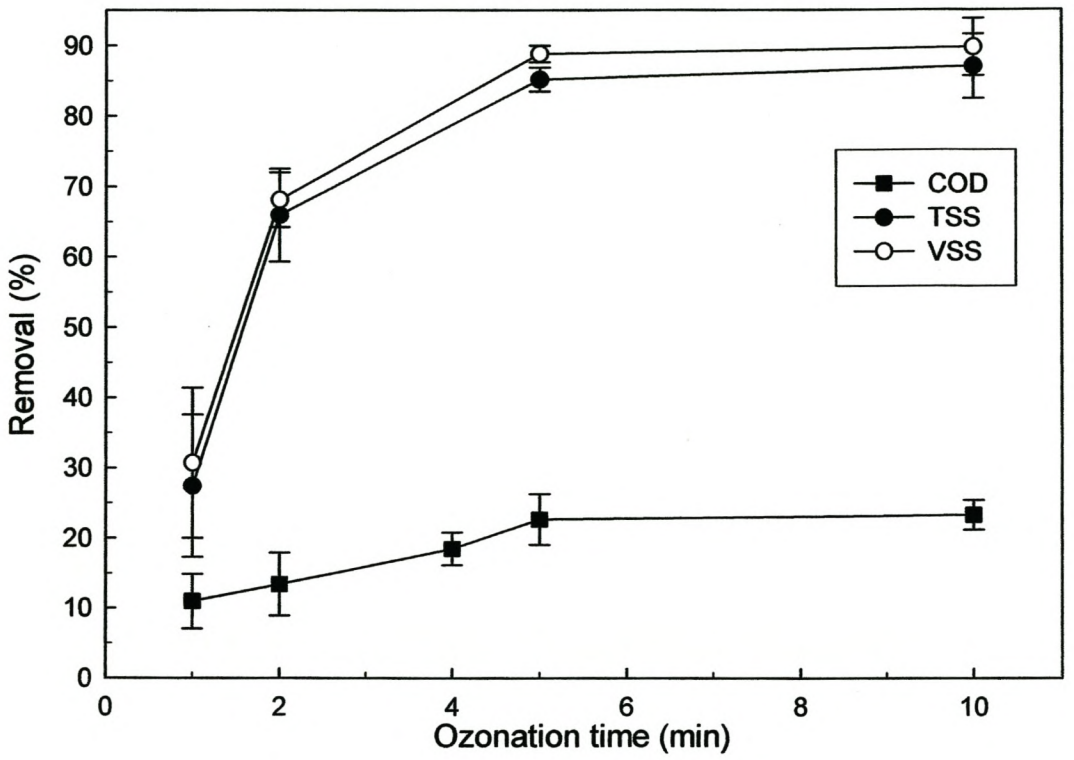

Figure 3. The influence of ozone treatments on the COD, TSS and VSS of UASB treated F-FR cellar effluent (COD = 810 mg.L^{-1}; TSS = 61 mg.L^{-1}; VSS = 51 mg.L^{-1}) (COD n = 6; TSS n = 4; VSS n = 4) (the standard deviation was used as the error-bar length).

23.3%. The COD reduction after 5 min ozonation was lower than the 30% reduction reported by Sigge *et al.* (2002) while ozonating the effluent from an UASB treating a similar cellar wastewater. These authors used a granular activated carbon contacting column in combination with the ozonation process and that may explain the higher COD removal.

Post-ozonation of the UASB reactor effluent also led to a reduction in both the TSS and VSS (Fig. 3). As was found with the COD reduction, the most degradation was achieved within the first 5 min of ozonation. After the 5 min treatment, the TSS was reduced from an average of 60.5 to 9.0 $mg.L^{-1}$ (85.1%). In the same period the VSS was reduced from an average of 50.5 to 5.6 $mg.L^{-1}$ (88.9%). With the 10 min ozonation treatment, the TSS and VSS were reduced only by 86.6 and 89.5%, respectively. For economical reasons, a 5 min post-ozonation treatment thus appears to be the most feasible option.

Ozonation of the UASB reactor effluent led to an increase in the effluent pH with the highest increase after only a 1 min treatment (Fig. 4). The pH increase was expected as ozone decomposes in water to produce oxygen and free hydroxyl radicals (Hammer & Hammer, 1996), which could possibly react with carboxyl groups of compounds in the wastewater. This needs to be further researched. Increased ozonation was also found to give a decrease in colour, as was evident from the decrease in absorption at 288 nm (Fig. 4). After 5 and 10 min ozone treatments the colour (absorption) of the effluent was reduced by 71 and 74%, respectively. In comparison, Athanasopoulos & Athanasopoulos (1998) only achieved a colour reduction of 74% after two hours ozonation in the treatment of an UASB treated "currant" effluent. The large difference in treatment time could be explained by the ozone production of only 300 $mg.h^{-1}$, compared to 17 500 $mg.h^{-1}$ as used in this study. The results obtained are, however, in accordance with the colour reductions of 68% and 80% for a 5 and 10 min ozonation treatment, respectively, reported by Sigge *et al.* (2002) while treating a similar cellar wastewater.

Treatment D

The pre-treatment of cellar wastewater followed by an UASB treatment (Treatment D: O_3 + UASB) led to a total COD reduction of 84 to 89%. The effect of ozone pre-treatment on the efficiency of an UASB reactor over a study period of

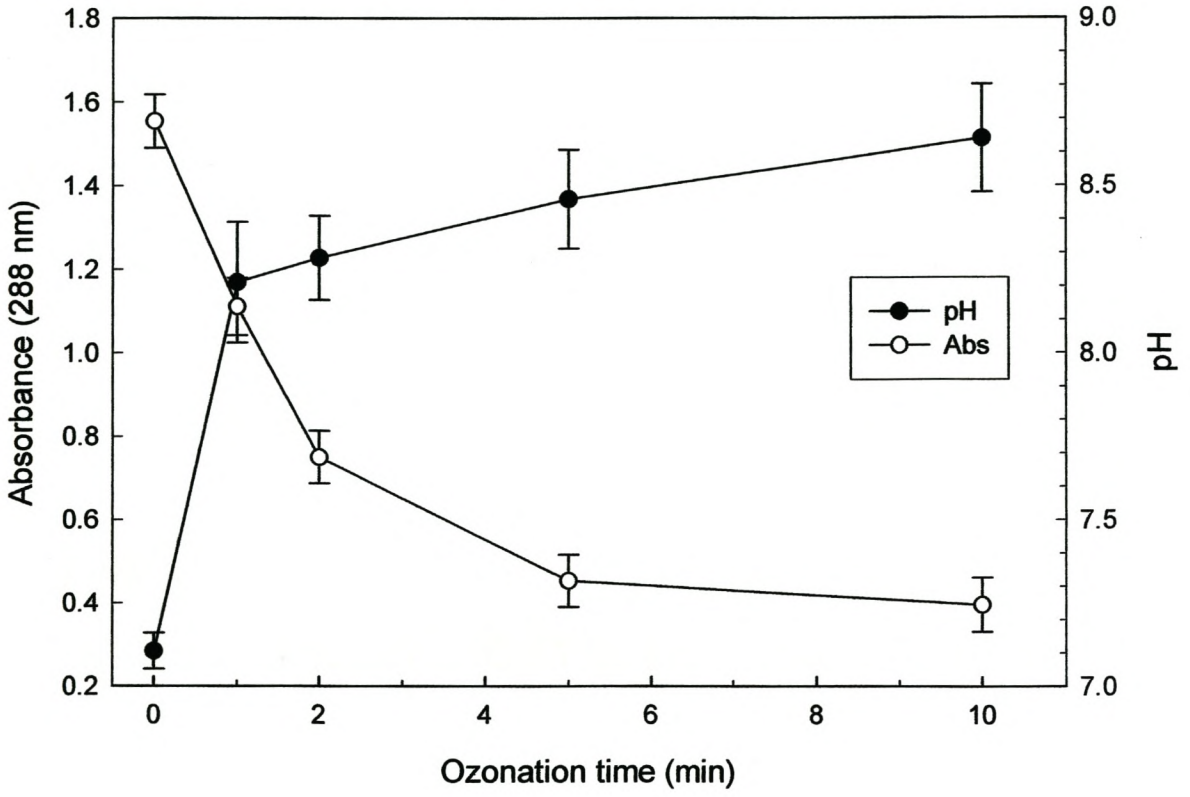

Figure 4. The influence of ozone post-treatments on the pH and absorbance (at 288 nm) of an UASB treated R-RF cellar wastewater (pH n = 7; absorbance n = 3). (the standard deviation was used as the error-bar length).

74 d is shown in Fig. 5. During the first 24 days of operation the reactor was adjusted to a new cellar wastewater (BK wastewater). Previously this UASB reactor treated R-RF wastewater at a substrate COD load of 5 400 $mg.L^{-1}$, substrate pH of 6.0 and HRT of 17.6 h. The low HRT led to reactor instability in terms of COD removal and effluent pH, and also granule wash-out (Experimental study III, Chapter 3 of this thesis). Accordingly, the HRT was re-set to 24 h and the OLR adjusted to 4 kg $COD.m^{-3}d^{-1}$ in order to again stabilise and improve reactor performance. The substrate pH was also increased to 7.0. The reactor was stable for 10 days (effluent pH = 6.8 and COD removal efficiency = 74%) before the substrate change to BK wastewater was made at the start of this part of the study (Treatment D).

The UASB reactor treated the BK wastewater at a substrate COD of 4 000 $mg.L^{-1}$ (minimum of 3 461 and maximum of 4 331 $mg.L^{-1}$), HRT of 24 h and pH poised at 7.00 (Fig. 5). From day 1 to 5 the COD removal increased from 51 to 62% and then varied between 61 and 67% during the period between d 5 and 24 (Fig. 5). The COD removal efficiency was extremely low as the reactor previously treated other cellar wastewater batches, and had reached COD removal efficiencies of 85% (R-RF wastewater). During the acclimatisation period (days 1 – 24) the VFA-concentration remained high and stabilised at an average of 760 $mg.L^{-1}$. Relatively little biogas, an average of 1.4 $L.d^{-1}$, was produced. The percentage methane in the biogas as measured on days 5 and 17 was 30 and 26%, respectively. The average methane production yield coefficient was calculated as 127 mL $CH_4.g^{-1}$ COD. The alkalinity remained above the lowest value (1 000 mg $CaCO_3L^{-1}$) for satisfactory digestion (Tchobanoglous & Burton, 1991).

On day 24, pre-ozonated cellar wastewater was introduced to the reactor, replacing the raw diluted wastewater substrate. Pre-ozonation led to a 20% reduction in the COD of the cellar wastewater, which had an initial COD of *ca.* 8 000 $mg.L^{-1}$. The ozonated wastewater was subsequently diluted to the substrate COD of *ca.* 4 000 $mg.L^{-1}$ to which the reactor was acclimatised.

Initially the change in substrate led to the COD removal efficiency being lowered to 58% (days 25 – 27) (Fig. 5). The decrease was coupled to an increase in the VFA-concentration to 998 $mg.L^{-1}$. By day 29 the reactor recovered to its

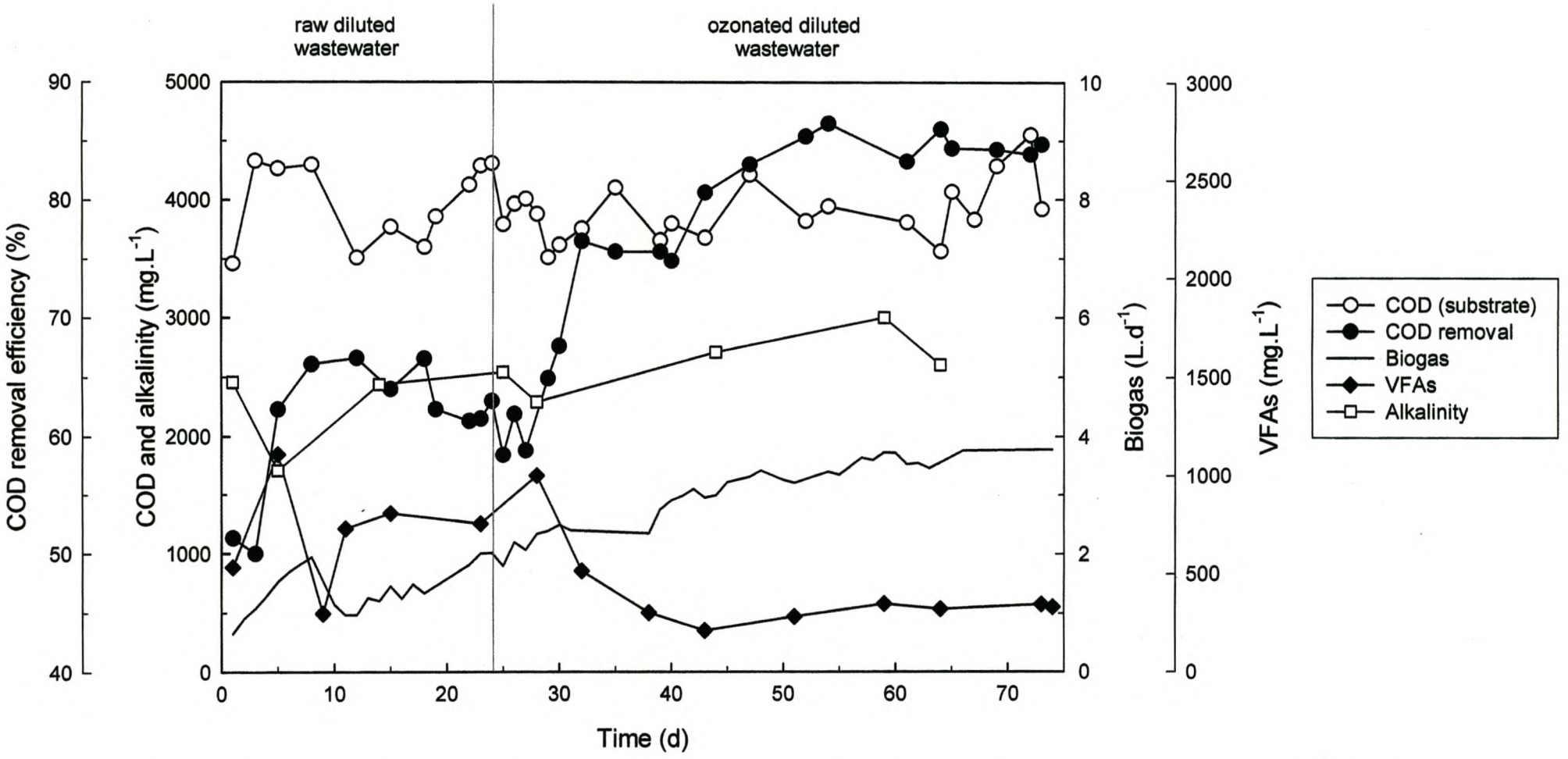

Figure 5. COD removal efficiency parameters of the UASB bioreactor treating a raw diluted BK cellar wastewater and a pre-ozonated diluted BK cellar wastewater.

best performance in terms of COD removal efficiency (65%) prior to the substrate change. The biogas production by this stage had increased to 2.3 $L.d^{-1}$. A significant improvement in COD removal efficiency occurred after day 29, with an increase up to 76%, by day 32. By day 43 the COD removal efficiency had further improved to 81% and by day 47, it was 83%. The reactor was now considered stabilised in terms of COD removal efficiency, which varied between 83 and 86% for the remainder of the study until day 74.

Coupled to the increased COD reduction after day 24, the VFA content of the UASB effluent decreased steadily after day 27 (Fig. 5). The VFA concentration varied between 221 and 348 $mg.L^{-1}$ and alkalinity remained above 1 375 mg $CaCO_3.L^{-1}$ for the remainder of the study (d 24 to 74), highlighting the increased performance of the UASB reactor. By day 74 the biogas production had also increased to 3.8 $L.d^{-1}$ (Fig. 5) and the percentage methane in the biogas was 36%. The average methane production yield coefficient was now calculated as 402 mL $CH_4.g^{-1}$ COD. Other authors also found the yield coefficient for methane to improve when using ozonation prior to AD (Benitez *et al.*, 1999; Weemaes *et al.*, 2000; Martin *et al.*, 2002). Benitez *et al.* (1999) and Martin *et al.* (2002) however, did not find an improvement in reactor performance in terms of COD removal efficiency when using an ozonated substrate for AD.

Treatment E

Ozonation of the effluent from the UASB reactor while treating pre-ozonated BK wastewater (Treatment E: O_3 + UASB + O_3) led to the COD , TSS and VSS being reduced (Fig. 6). The COD, TSS and VSS reductions for the UASB effluent (COD = 634 $mg.L^{-1}$; TSS = 40.3 $mg.L^{-1}$; VSS = 35.0 $mg.L^{-1}$) after a 5 min ozonation period was 16, 75 and 78%, respectively. The 5 min post-ozonated effluent gave final COD, TSS and VSS concentrations of 531, 10.1 and 7.6 $mg.L^{-1}$, respectively. Extending the post-ozonation treatment to 10 min, only led to slight improvements in reduction of COD, TSS and VSS.

As was found the case in Treatment C, the pH of the post-ozonated UASB bioreactor effluent rose with extending the ozonation time (Fig. 7). The colour value (absorbance at 288 nm) was also lowered with a lengthening in ozonation time, from 1.10 to a final absorbance of 0.30 after 10 min ozonation. This was lower than the absorption of 0.40 achieved in treatment C. The colour reduction of

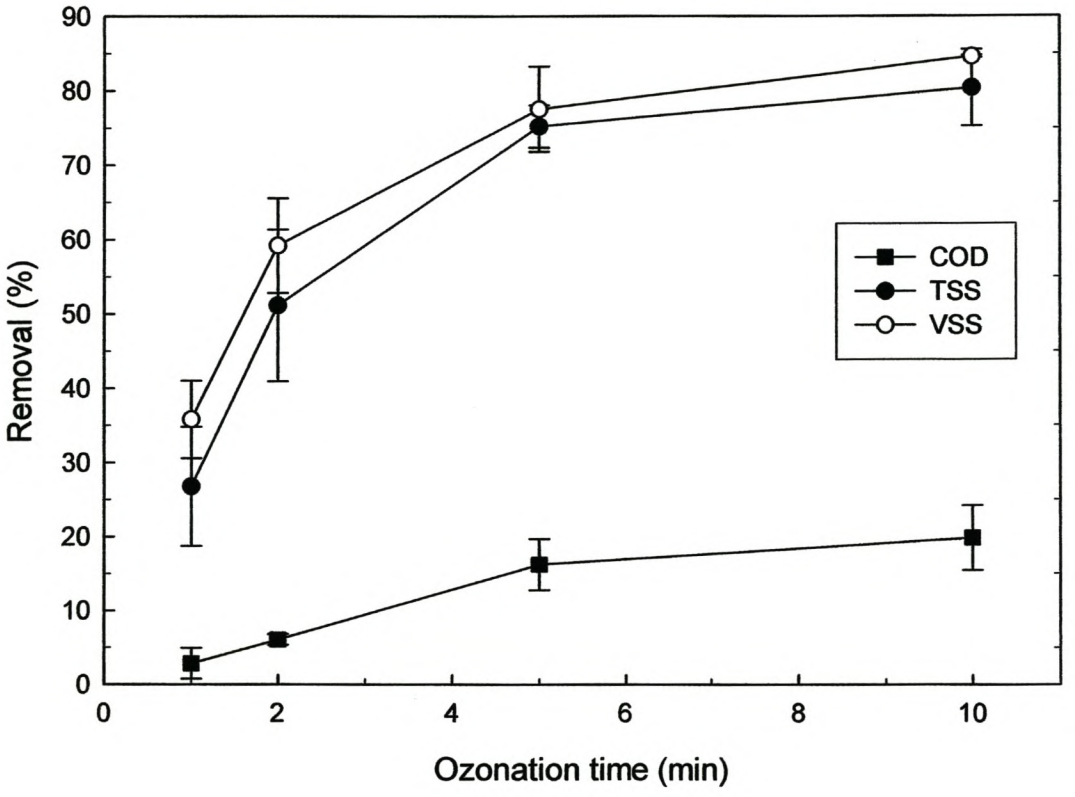

Figure 6. The influence of a post-UASB ozonation on the COD, TSS and VSS of an UASB reactor effluent (COD = 634 $mg.L^{-1}$; TSS = 40.3 $mg.L^{-1}$; VSS = 35.0 $mg.L^{-1}$) , treating pre-ozonated BK cellar wastewater (COD n = 6; TSS n = 4; VSS n = 2) (the standard deviation was used as the error-bar length).

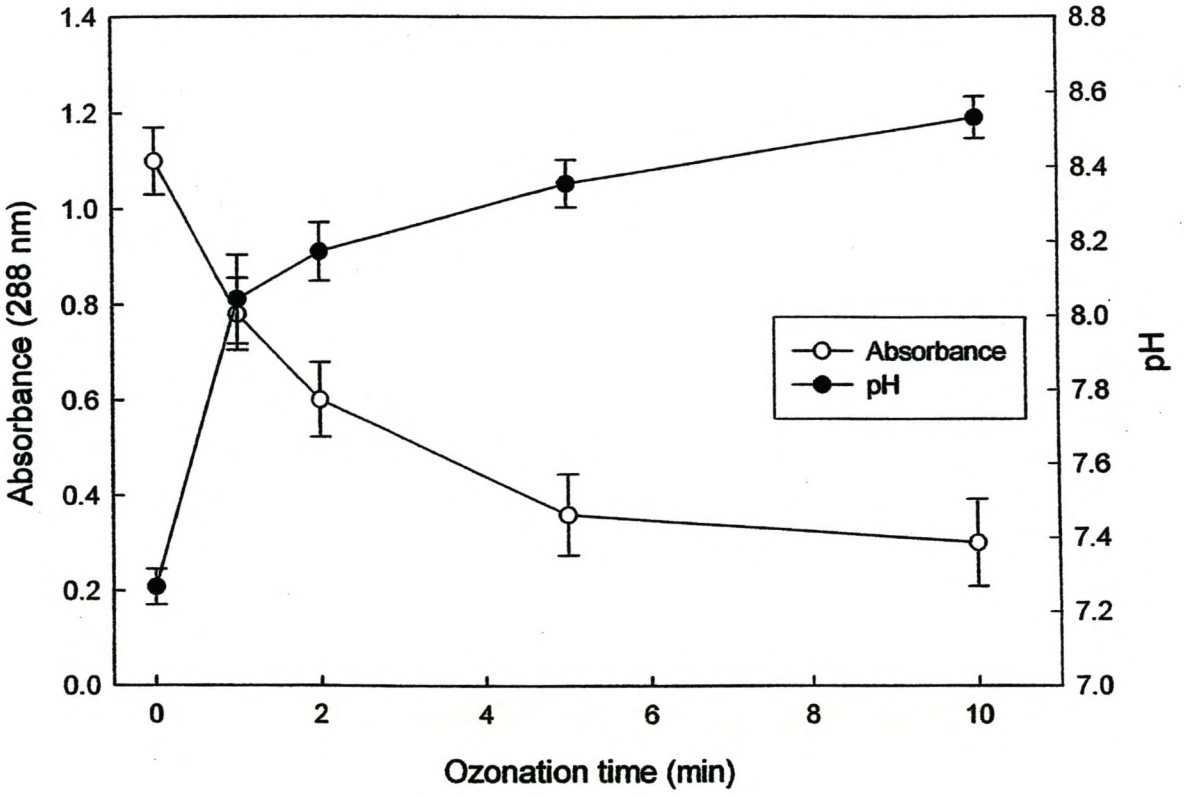

Figure 7. The influence of a post-UASB ozonation on the pH and absorbance of an UASB reactor effluent, treating pre-ozonated BK cellar wastewater (pH n = 5; absorbance n = 5) (the standard deviation was used as the error-bar length).

73% for the UASB treatment and post-ozonation step of Treatment E, did however, compare well to the results found in Treatment C (UASB + O_3) of 74%.

Conclusions

From the data obtained in this study it was concluded that 5 min would be the optimal time in terms of efficiency and cost for the pre- and post-ozonation treatments combined with UASB treatment. Ozonation of untreated wastewater (COD = 8 000 $mg.L^{-1}$; TSS = 1 180 $mg.L^{-1}$; VSS = 1 123 $mg.L^{-1}$) alone for 5 min (Treatment A: 5 min O_3) led to reductions in COD, TSS and VSS (Fig. 8).

The UASB treatment alone (Treatment B) was more effective in reducing COD, TSS and VSS. The reduction was, however, improved by a 5 min. post-ozonation treatment, resulting in a complete COD reduction for Treatment C (UASB + 5 min O_3) of 88%, compared to 85% for UASB treatment alone and 20% for a 5 min ozone treatment (Fig. 8). The total reduction of TSS for Treatment C (UASB + 5 min O_3) was 97% compared to 80% for UASB alone and 73% for a 5 min ozone treatment alone. The reduction for VSS was 98% (Fig. 8) compared to 81% for UASB alone and 73% for ozone treatment alone.

The total reduction when using the pre-ozonation UASB combination treatment (Treatment D: 5 min O_3 + UASB) was slightly less effective than Treatment C (UASB + 5 min O_3). The COD was reduced by an average 86% and TSS and VSS were both reduced by 95%, compared to the 88, 97 and 98% for COD, TSS and VSS removal, respectively, for treatment C. It was however, still more effective than using UASB treatment alone.

The most efficient reduction was obtained when using Treatment E (5 min O_3 + UASB + 5 min O_3). The total TSS and VSS reduction was 99% for both (Fig. 5) and the COD reduction for this treatment process was 89% (Fig. 8). Although legal limits for discarding into a natural resource were not quite met, significant progress was made in reducing COD levels.

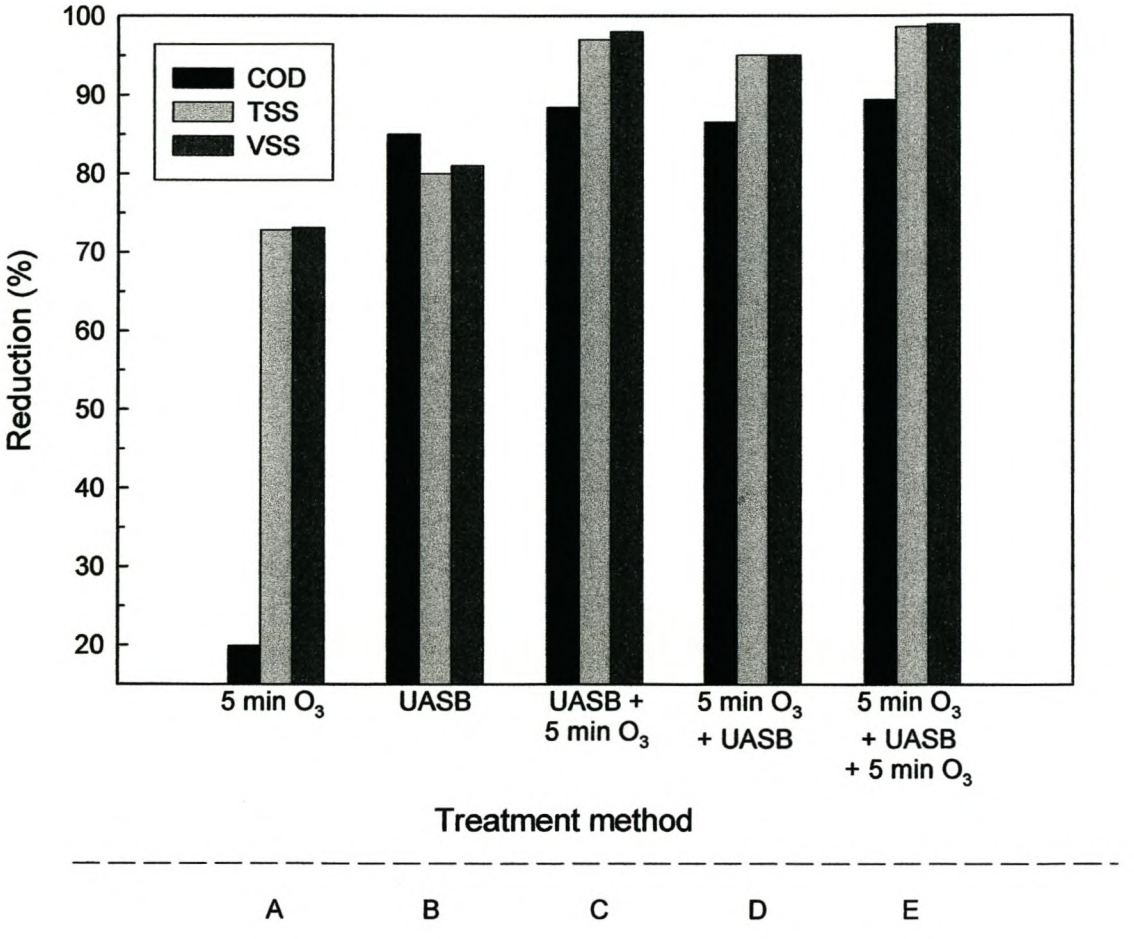

Figure 8. COD, TSS and VSS removal during various ozone/UASB treatment combinations for cellar wastewater degradation.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Background

South Africa, a water limited country is experiencing increasing problems with water scarcity and quality. Population growth and issues of social and economic development further aggravates the problem. The wine industry significantly contributes to water demand and subsequent pollution of the limited water resource (Van Schoor, 2000). Cellars produce wastewater throughout the year, which varies in organic load and pH according to the activities of the season (Bezuidenhout *et al.*, 2002). Current legislation requires cellars to implement a waste management system (Hayward *et al.*, 2000), which must place strong emphasis on treatment prior to disposal.

Anaerobic digestion (AD) using UASB technology has been shown to be successful in the treatment of wastewaters from the food, beverage, wine and distillery industries. Compared to aerobic treatment, AD has several advantages but although very efficient, set legal final standards for disposal cannot always be reached by AD technology alone. Thus it is observed that a combination with a chemical treatment, such as oxidation by ozonation, may be used to enhance process efficiency. As a pre-treatment, ozone is known to enhance the biodegradability of the raw substrate (Benitez *et al.*, 1999; Martin *et al.*, 2002). Ozonation may lead to the breakdown of large recalcitrant molecules into smaller molecules that can be utilised by the anaerobic populations and accordingly result in enhanced efficiency of UASB technology. However, it is also known that depending on the specific compound and ozonation time, the breakdown products of ozonation may become more inhibitory than the initial products itself (Andreozzi *et. al.*, 1998) and it is important that this should be evaluated before implementing ozonation as a treatment option. Similarly ozonation as a post-treatment can be seen as a final polishing step prior to disposal which may lead to the pollution levels of final effluent being within legal set limits.

UASB treatment

In the research done in this study a laboratory-scale UASB bioreactor containing mixed anaerobic granules was acclimatised to nutrient and carbohydrate deficient cellar wastewater (Chapter 3). While feeding the raw diluted substrate at a pH set at 8.0 at a hydraulic retention time (HRT) of 24 h, reactor stability could not be reached. Sucrose additions to the raw substrate, increased substrate loads, heat-treatment of the raw substrate and the addition of isolated "natural" cellar effluent bacteria to facilitate digestion prior to AD, were all fairly unsuccessful in stabilising the UASB in terms of COD removal efficiency. When the substrate pH was lowered to 7.5, stable-state conditions were however, achieved. It is possible that the higher substrate pH led to a build-up of salts in the reactor, which in turn led to reactor instability.

Cellar wastewater batches obtained during the collection period (vintage and non-vintage periods) exhibited variation in pH (3.6 to 6.3) and COD (3 500 to 10 200 $mg.L^{-1}$) values. Since the cost of neutralising the substrate to pH 7.5 could be excessive, it was deemed necessary to find the lowest efficient operational pH of the reactor. The reactor population was in a step-wise regime acclimatised to a lower substrate pH, with the lowest efficient operational substrate pH being found to be 5.7 for a substrate with COD less than 5 000 $mg.L^{-1}$ (COD removal = 88%). As the volatile fatty acid (VFA) concentration and effluent pH remained above 400 $mg.L^{-1}$ and below 7.0, respectively when substrate at this pH was treated, it was suspected that further pH reductions would have led to reactor failure.

As mentioned above, cellar wastewater did occasionally exhibited high organic loads in terms of COD (> 10 000 $mg.L^{-1}$). It was thus also important to find the highest possible load the UASB reactor would be able to treat effectively in the shortest possible time. The lowest efficient operational HRT and corresponding OLR were found to be 19.7 h and 9.75 kg $COD.m^{-3}d^{-1}$, respectively, when treating a cellar wastewater substrate at a pH of 6.0 (COD removal efficiency = 84%). Further decreases in HRT led to severe granule wash-out and irreparable prolonged instability of the reactor in terms of COD removal, effluent pH and alkalinity.

Ozonated cellar wastewaters

The possibility of ozonation as a pre-treatment to AD was considered, as it is known that ozonation can improve substrate biodegradability of substrates (Gottschalk *et al.*, 2000) and, therefore, enhance the AD process when used as a pre-treatment to AD (Benitez *et al.*, 1999; Martin *et al.*, 2002). Accordingly three dominant strains were isolated from raw cellar wastewaters and identified as *Acinetobacter haemolyticus*, *Burkholderia cepacia* and *Cryseomonas luteola* strains (Chapter 3). These strains were grown in sterile cellar wastewater and then inoculated into sterile cellar wastewater substrates from both the vintage and non-vintage periods (Chapter 4). The following growth substrates were prepared: controls, CON(A) and CON(B); substrates supplemented with nutrients, NUT(A) and NUT(B); and ozonated substrates, OZ(A) and OZ(B). The growth of the various isolates was monitored at 35°C.

All the isolates grew well in the control substrates from both the vintage and non-vintage periods leading to increases of at least 1.5 log cycles. The addition of nutrients to the substrate from the non-vintage period enhanced the growth of all the isolates. The exponential-phases were lengthened from 6 h for all the isolates in the control substrate to 12 h for *A. haemolyticus* and at least 24 h for *B. cepacia* and *C. luteola* in substrate NUT(A). In contrast, the addition of the same nutrient concentrations to the substrate from the vintage period led to a decreased growth than found in the control substrate from the same wastewater batch. Ozonation of the substrates for 10 min at a rate of 17.7 $g.h^{-1}$ led to a slight increase in growth for all the isolates in substrate OZ(B). It was thus found that ozonation did in fact improve the biodegradability of the substrate. However, the opposite was observed for substrate OZ(A) where ozonation led to the wastewater (non-vintage) becoming inhibitory and even detrimental to bacterial growth.

From the results of this study, it was concluded that ozonation of cellar wastewater substrates could either improve biodegradability or lead to the substrate becoming inhibitory to bacterial growth. It has been reported that ozonation may lead to the formation of recalcitrant products that are higher in toxicity than the original substances (Andreozzi *et al.*, 1998). Thus, it is recommended that further studies specifically evaluating the substrate composition

could be of value in explaining why the non-vintage cellar wastewater substrate became inhibitory to the growth of the selected bacterial isolates.

Ozonation as pre- and post-treatment to UASB-treatment

Although the data obtained in this study showed that AD was effective in lowering the COD of the cellar wastewaters (Chapter 3), the 75 $mg.L^{-1}$ standard set by legislation for final disposal in a natural water resource, were not met (Anon. 1999). Thus, ozonation was evaluated as both a pre- and post-treatment to UASB treatment in an attempt to reach the standard or at least to lower the organic load in the wastewater to a value as close as possible to the standard (Chapter 5).

It was found that ozonation alone for 5 min at a concentration of 73 $mg.L^{-1}$, led to a 20% reduction of the COD and a 73% reduction for both the total suspended solids (TSS) and volatile suspended solids (VSS) of cellar wastewater. Polyphenols, which are known to be toxic to the methanogenic bacteria (Hamdi, 1993), were reduced by at least 73% after only a 2.5 min ozone treatment. A 5 min ozone treatment was found to be the optimum ozonation time, as further ozonation only led to slight increases in the reduction of the efficiency parameters.

When ozone was applied as a post-treatment to the effluent from an UASB the total reduction for COD, TSS and VSS were 88, 97 and 98%, respectively, resulting in a final wastewater substrate with a COD of 648 $mg.L^{-1}$. This was a great improvement as the UASB treatment alone led to a final COD value of 810 $mg.L^{-1}$. Post-ozonation also led to a 71% decrease in the UASB reactor effluent colour. As for ozone treatment alone, the optimal ozonation time for post-ozonation was found to be 5 min.

Pre-ozonation was slightly less successful than post-ozonation (Chapter 5) with a COD reduction of 86%. The pre-ozonation process did, however, improve the performance of the UASB reactor, which only achieved a 67% COD reduction prior to pre-ozonation. The biogas production of the UASB reactor also increased from 1.4 to 3.8 $L.d^{-1}$ and the methane yield coefficient from 127 to 402 mL $CH_4.g^{-1}$ COD. The most successful treatment process was found to be a combination of both a pre-, UASB and post-ozonation treatment resulting in a final wastewater with a COD of 530 $mg.L^{-1}$.

Concluding remarks

The use of a combined pre- and post ozonation-UASB treatment process was found to be feasible for the treatment of carbohydrate deficient cellar wastewater. It was possible to optimise the UASB bioreactor into treating the cellar wastewater at a pH of 5.73 (HRT = 24 h and substrate COD<5 000 $mg.L^{-1}$). The lowest operational HRT and OLR were found to be 19.7 h and 9.75 kg $COD.m^{-3}d^{-1}$, respectively. This is of great importance as cellar wastewaters are often very acidic (pH = 3.6) and can have high organic contents (COD = 10 000 $mg.L^{-1}$).

Although the combined process was successful, the legislative standard for disposal into a natural resource was not reached. With slight refinements, other forms of disposal methods, such as irrigation, might now be an option. The combination of ozonation with other oxidants such as H_2O_2 must still be investigated so as to try to further enhance the ozonation process. It was, furthermore, important to note that not all cellar wastewaters reacted positively on ozonation and some even became inhibitory and detrimental to bacterial growth. Future research should include the compositional analyses of cellar wastewater so as to determine the compound(s) responsible for this action. A complete study should also be conducted on pilot- and full-scale to determine the actual feasibility of the combined process for the treatment of cellar wastewaters on industrial scale.

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